Reactions of Steroidal Epoxides with Strong Organic Bases and An Investigation into the Syntheses of Some Labelled Pregnane Derivatives

by

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St. Catharines, Ontario

ABSTRACT

Reactions of 5,6- and 4,5-epoxycholestane derivatives with strong bases were investigated.

Epoxidation of 3α-acetoxycholest-5-ene also gave a new compound along with the anticipated epoxides. Interconversions of the latter were observed. Some possible mechanisms of its formation and rearrangements have been proposed.

No reaction was observed with any of the 5,6- and 4,5-steroidal epoxides employed in the present study, using potassium tertiary butoxide under refluxing conditions. n-Butyllithium reacted only with 5,6-epoxycholestanes bearing a ketal moiety at the C3 carbon. Opening of the ketal group was observed with n-butyllithium in the case of a β-epoxide. The reaction was also investigated in the absence of epoxide functionality. A possible mechanism for the opening of ketal group has been proposed.

Lithium diethylamide (LDEA) was found effective in rearranging 5,6- and 4,5-epoxides to their corresponding allylic alcohols. These rearrangements presumably proceed via syn-eliminations, however the possibility of a corresponding anti-elimination has not been eliminated.

A substituent effect of various functional groups (R = H, OH, OCH_2CH_2O) at C3 has been observed on product distribution in the LDEA promoted rearrangements of the corresponding epoxides.

No reaction of these epoxides was observed with lithium diisopropylamide (LDA).

In the second part of the project, several attempts were made towards the synthesis of deoxycorticosterone-17,21,21-d_3, a compound desirable for the 21-dehydroxylation studies of deoxycorticosterone. Several routes were investigated, and some deuterium labelled pregnane derivatives were prepared in this regard. Microbial 21-hydroxylation of progesterone-17,21,21,21-d_4 by A. niger led to loss of deuterium from C21 of the product. An effort was made to hydroxylate progesterone microbially under neutral condtions.

ACKNOWLEDGEMENTS

I would like to express my appreciation to Dr. H. L. Holland for his guidance, boundless patience, encouragement, enthusiasm and optimism during the course of this work.

My grateful thanks are also extended to Dr. J. M. Miller and to Dr. M. S. Manocha (Department of Biological Sciences) for many valuable discussions. I would also like to thank Mr. Tim Jones for recording mass and NMR spectra, and the technical staff of Brock University, especially Mr. Vandenhoff. I am also thankful to B. Sayer of McMaster for recording ^{2}H NMR spectra, as well as to Dr. R. E. Lenkinski of the University of Guelph for ^{1}H NMR, ^{2}H NMR and ^{13}C NMR spectra.

I am indebted to my fellow graduate students and lab colleagues who contributed to making my stay at Brock an enjoyable one. I must express my gratitude to Dr. P. Ch. Chenchaiah and Dr. S. S. Gandhi for their invaluable help with proof reading. I want to express my deep appreciation to Afzal and Almas for their encouragement and moral support.

Finally, my thanks to Miss Janet Hastie for deciphering my scrawls and translating them into a neatly typed manuscript.

To

my parents

for their patience

"...the organic chemist...should not be surprised if his predictions in a new situation turn out to be erroneous. It is frequently difficult to anticipate all the factors which may be critical in any given situation."

A. Corwin and M. Bursey

(from "Elements of Organic Chemistry")

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The steroids comprise a class of compounds which are based on the perhydrocyclopentenophenanthrene nucleus (Fig. 1). This group encompasses a wide range of biological important molecules including the sterols, bile acids, sex hormones, adrenocortical hormones, cardiac glycosides, sapogenins and some alkaloids.

The biosynthetic precurosr of the steroids is the two carbon acetate, which is a known precursor of a great many natural products. Discovering the way in which a steroid nucleus of rigidly defined stereochemistry arises from this simple compound has been the subject of much research.[1]

The steroid nomenclature is based upon the nature of the hydrocarbon framework from which the steroid is derived. The most common of these hydrocarbon skeletons are presented in Figure 2. The numbering scheme employed in the systematic nomenclature of these compounds is given in Figure 3. As can be readily seen from a stereochemical representation (Fig. 4), the 5α-steroid nucleus is nearly planar. Substituents which project above the plane of this ring system are labelled β, while those projecting below are labelled α.

After the isolation of cholesterol (1), the most dramatic expansion of steroid chemistry came with this discovery of the sex hormones in 1931-1935 and of the adrenalcortical hormones in 1935-1938.

In fact, steroid chemistry before 1950 is a story of remarkable achievements with primitive tools. The unravelling of steroid molecular

Figure 1

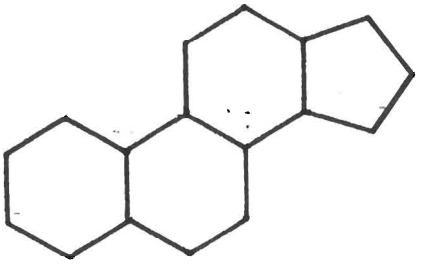
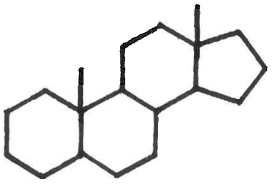
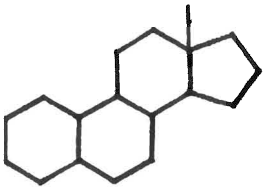


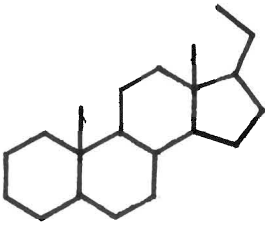
Figure 2



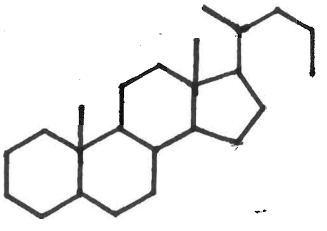
androstane



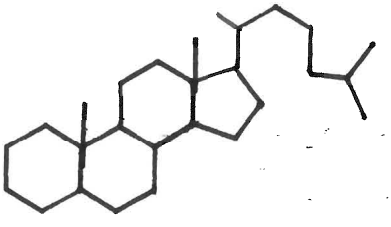
estrane



progestane



cholane



cholestane

Figure 3

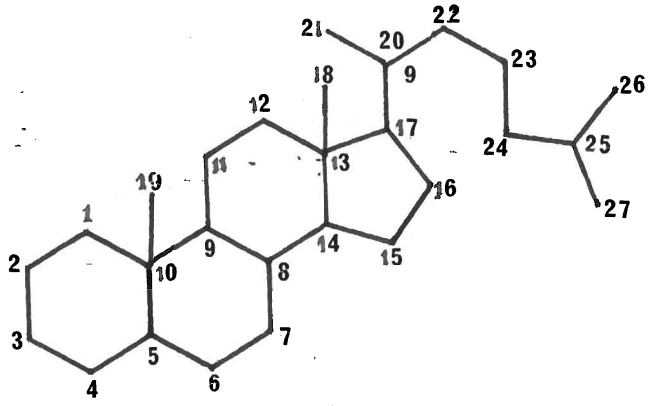
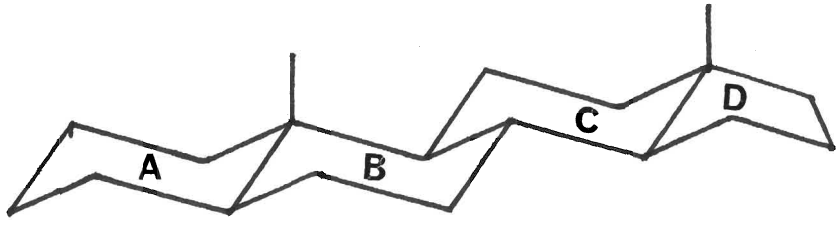


Figure 4



structures, their interconversion, and their syntheses during this period, demand the highest admiration for the early generations of steroid chemists, whose approach had to be largely empirical. Today's techniques of spectroscopy and chromatography were unknown or in their infancy, and the conformational aspects of steroids had not been realized. Very many steroid reactions were documented, but few were really understood. The study of reaction mechanisms lay in a specialized field which hardly impinged upon the chemistry of "Natural products".

Nearly ninety years ago, Sachse[2] laid the foundation of conformational analysis by proposing that a cyclohexane ring must exist in one of two unstrained forms, but the detailed shape of a ring must have seemed to most chemists hardly more than a matter for curiosity, it certainly excited little interest among organic chemists for several decades. In 1950, however, Barton[3] demonstrated the key role of conformation features in controlling many of the properties and reactions of alicyclic molecules, and was able to fit experimental data of many kinds into a single consistent pattern. Within a short span of time alicyclic chemistry, reaction kinetics and mechanisms, and a variety of other features of organic compounds, became firmly linked within the framework of conformational analysis. Indeed Barton's first enunciation of the role of the conformational features in determining the properties and reactions of organic compounds drew examples largely from the steroid field.[3]

With the arrival of conformation ideas, and Barton's demonstration that the steroids have a chemistry which is not only logically predictable in many respects, but also intimately related to the general interests of

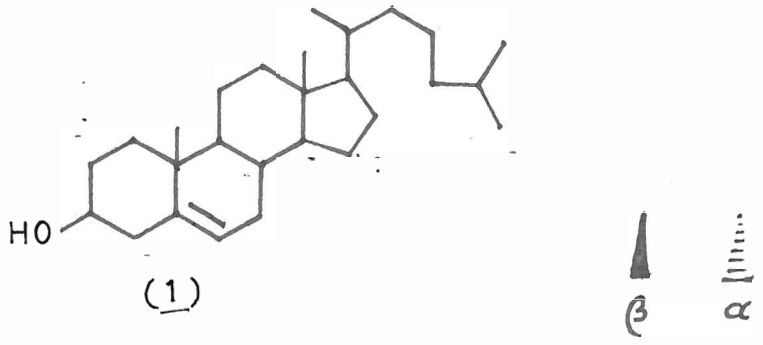
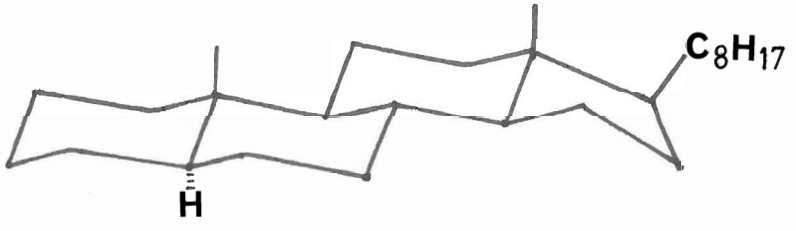
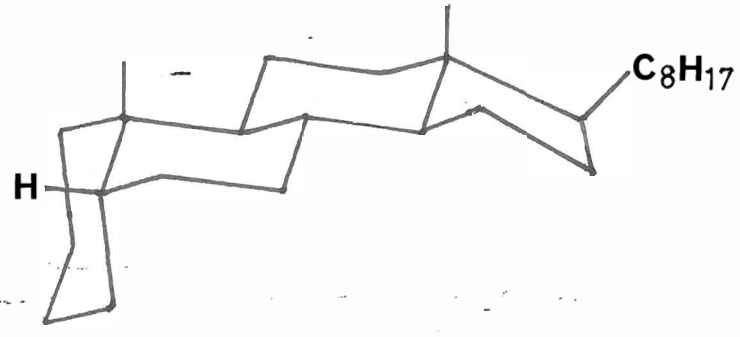


Figure 5



5α-Cholestane (A/B trans)

Figure 6



5β-Cholestane (A/B cis)

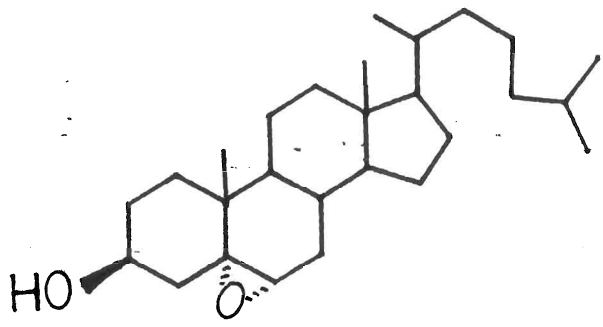
organic and physical organic chemists, has resulted in a surge of enthusiasm for the study of steroids which is now in full flood. This work has contributed much to the development of modern concepts of organic reactions by mechanisms from the idea first propounded in detail by Ignold.[4]

The widespread use of steroids in exploring the scope and mechanisms of new chemical reactions is now an established part of organic chemistry. Nevertheless, new reactions often lead to steroids with quite strange structural features and those occasionally discovered to exhibit significant physiological activity.

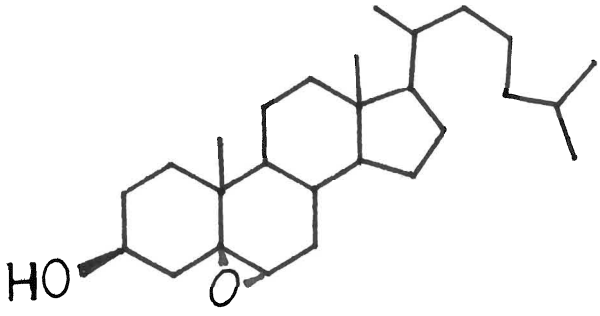
The present study deals with the steroids having cholestane skeleton. In 5α-cholestane (Fig. 5), all the ring functions are trans, while the only difference in 5β-cholestane (Fig. 6) is the A/B ring function which has cis stereochemistry. These rings then each include one bond axial to the other, making 5β-steroids generally less stable. All the six-membered rings normally have the more stable chair conformation, and prefer to react in this conformation, although in rare circumstances, where the transition state for a reaction is more readily accessible from a flexible conformation of the ring involved, the reaction proceeds slowly through the less stable "flexible" conformation even if present in very low concentration. The present study, to the best of our knowledge, is the first devoted to examining the base promoted reactions of steroidal epoxides with poorly nucleophilic, strongly basic reagents. This is an aspect of epoxide chemistry which has not shared in the extensive exploitation that this small heterocycle ring, in general, has enjoyed.

The steroidal epoxides are not only of chemical interest, but some of them have significant biological importance, for example, those of cholesterol and pregnenolone. Some suggestive aspects were reported with respect to the fact that cholesterol α-epoxide (2) among a number of endogenous epoxides,[5-7] might play a role as a systemic carcinogen. As early as 1969, it was demonstrated that cholesterol α-epoxide (2) given subcutaneously to rats and mice caused them tumours at injection sites.[8] Furthermore, later workers[9] reported a suggestive role of the cholesterol α-epoxide (2) as a chemical mediator in the induction of skin tumour in hairless mice irradiated by ultraviolet rays.

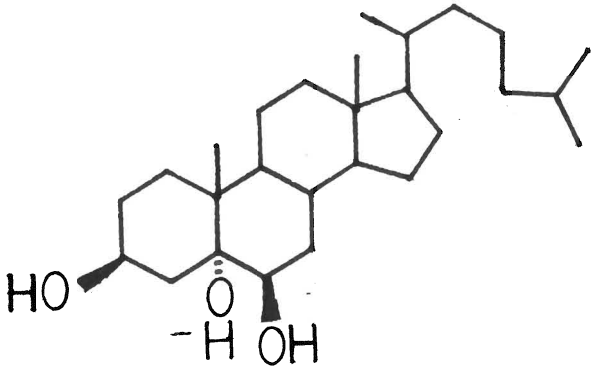
Very recently, cholesterol α-epoxide (2) has been demonstrated[10] to induce malignant transformation of cells in culture and form a covalent bonding to the bases of isolated DNA.[11] It is of interest to note that the hydrolysis product of both cholesterol α- and β-epoxide (3), the cholestane-3β,5α,6β-triol (4) is excreted in significantly higher quantity in the feces of patients suffering with ulcerative colitis and colon cancer.[12] Apart from its suspected carcinogenic activity, the α-epoxide was found at significantly high levels in sera of patients with high blood pressure, peptic ulcers and hypercholesterolemia[13] as well as in the organs of patients with Wolman's disease.[14] Bovine liver microsomes convert endogenous Δ^5-steroids to the corresponding 5α,6α-epoxides, 5β,6β-epoxides and 5α,6β-glycols in the presence of an NADPH generating system, ferrous ion and ADP.[15] The α and β epoxides, formed in 4:1 ratio, serve as intermediates and are hydrolysed by hepatic microsomes to their corresponding 5α,6β-glycols (Fig. 7). The hydrolysis of these epoxides is also most



(2)



(3)



(4)

probably non-enzymatic as non-enzymatic hydrolyses of several 5,6-epoxy steroids have been observed by Holland et al.[16] with Rhizopus arrhizus.

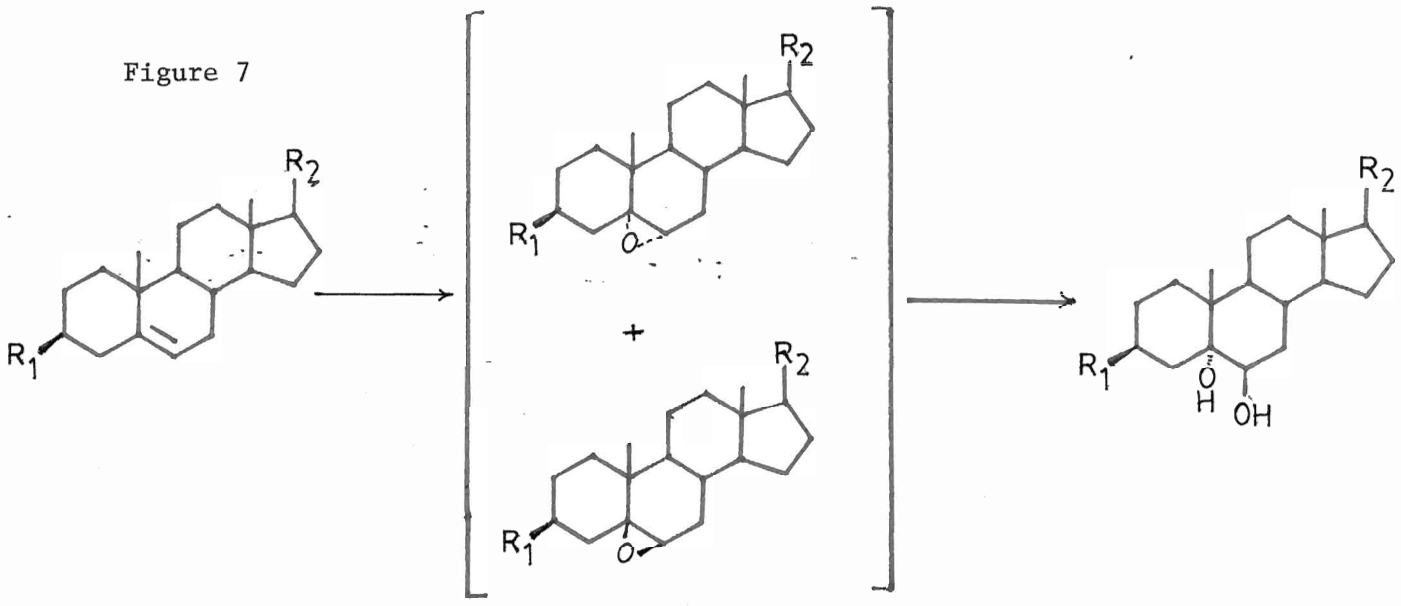
The chemistry of epoxides is dominated by the strained character of the three-membered ring consequent upon angles between the bonds. It is perhaps more accurate to regard the bonds as being "banana-shaped" as in cyclopropane,[17] where bonding is interpreted as the most economical compromise, energetically, between efficient overlap of atomic orbitals and contraction of interorbital angles from its optimum tetrahedral value of ∿109.5° to a value estimated as 104° (Fig. 8).

The strained character of epoxides is revealed in their ease of ring opening which leads to a variety of interesting reactions under the influence of proton acids, Lewis acids, nucleophilic reagents or strong bases by abstracting α, β or higher protons.

Acid-catalyzed reactions of epoxides probably display greater diversity of character than any other reactions in the steroid field. Hence quite minor structural variations can result in complete alteration of the reaction pathway. Since the acid-catalyzed reactions are the commonest reactions of these epoxides, therefore, it is worthwhile to mention them briefly.

The most detailed studies of steroidal epoxide reactions in the absence of external nucleophiles have employed boron trifluoride as a Lewis acid in a non-polar solvent, and are mechanistically well understood. Some of the boron trifluoride-catalyzed reactions of epoxy cholestanes[18,19] have been outlined in Figure 9. The unsymmetrical epoxides undergo C-O bond cleavage at the more substituted carbon atom and commonly give ketonic products by

Figure 7



Cholesterol $R_1 = OH$; $R_2 = C_7H_{18}$

Pregnenolone $R_1 = OH$; $R_2 = COCH_3$

Δ^5-Cholestene $R_1 = H$; $R = C_7H_{18}$

20-Methyl-pregnenolone $R_1 = OH$; $R_2 = CH(CH_3)_2$

Figure 8

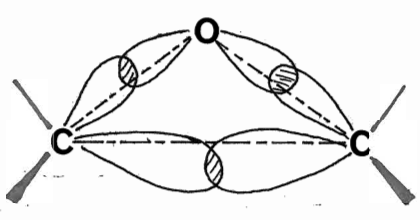
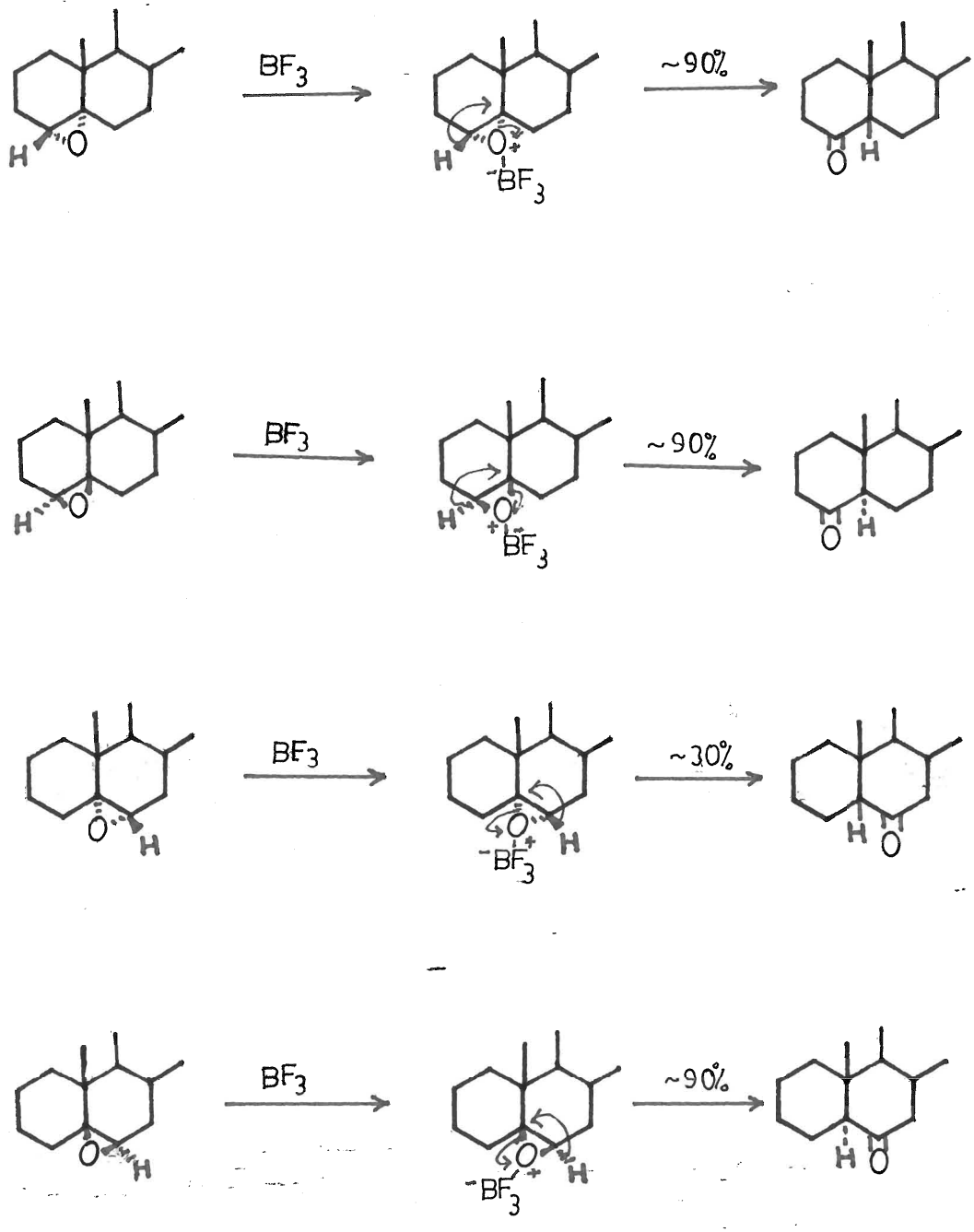


Figure 9



migration of a "hydride ion" to the positively charged centre, with retention of the configuration of the migrating group.[20,21]

Tetra-substituted epoxides have no overwhelming electronic preference for cleavage at a particular C-O bond. The four examples illustrated[22,23] in Figure 10 show that some other mode of control is operating, and this has been formulated in the "rule of diaxial cleavage".[24]

Attempts to predict, from precedent, the behaviour of any particular alicyclic epoxide with a Lewis acid have been complicated by the great diversity of known reactions, many of them undergo skeletal rearrangement and eliminations.

The A-nor-3β,5β-epoxide (5) reacts with BF_3 to give the fragmentation product, A-nor-cholest-3(5)-ene (6), with a little 5α-isopropyl ketone (7).[25] A possible rearrangement sequence is illustrated in Figure 11.

The rearrangements of 11α,12α epoxy steroids are profoundly influenced by 12β substituents. The 12β-phenyl tigogenin (8) reacted with BF_3 in a manner totally different from the 12β-methyl epoxide[26] (9). Reaction of 12β-methyl-11α,12α-epoxide (9) with BF_3 gave 11α-hydroxy-13α-methyl-C-nor-D-homo derivative (10) as the major product along with 11β-hydroxy-13α-methyl-C-nor-D-homo derivative (11) and 11-oxo-12α-methyl compound (12) (Fig. 12). The formation of (10) and (11) is depicted in Figure 13.

On the other hand, 3β-acetoxy-11α,12α-epoxy-12β-phenyl tigogenin (8) has been reported[27] to give three compounds (Fig. 14). All the main products arise from initial migration of the 13β-methyl group to C-12.[27] The resulting C-13 carbonium ion (13) reacted further in three different ways to give three products (Fig. 14).

Figure 10

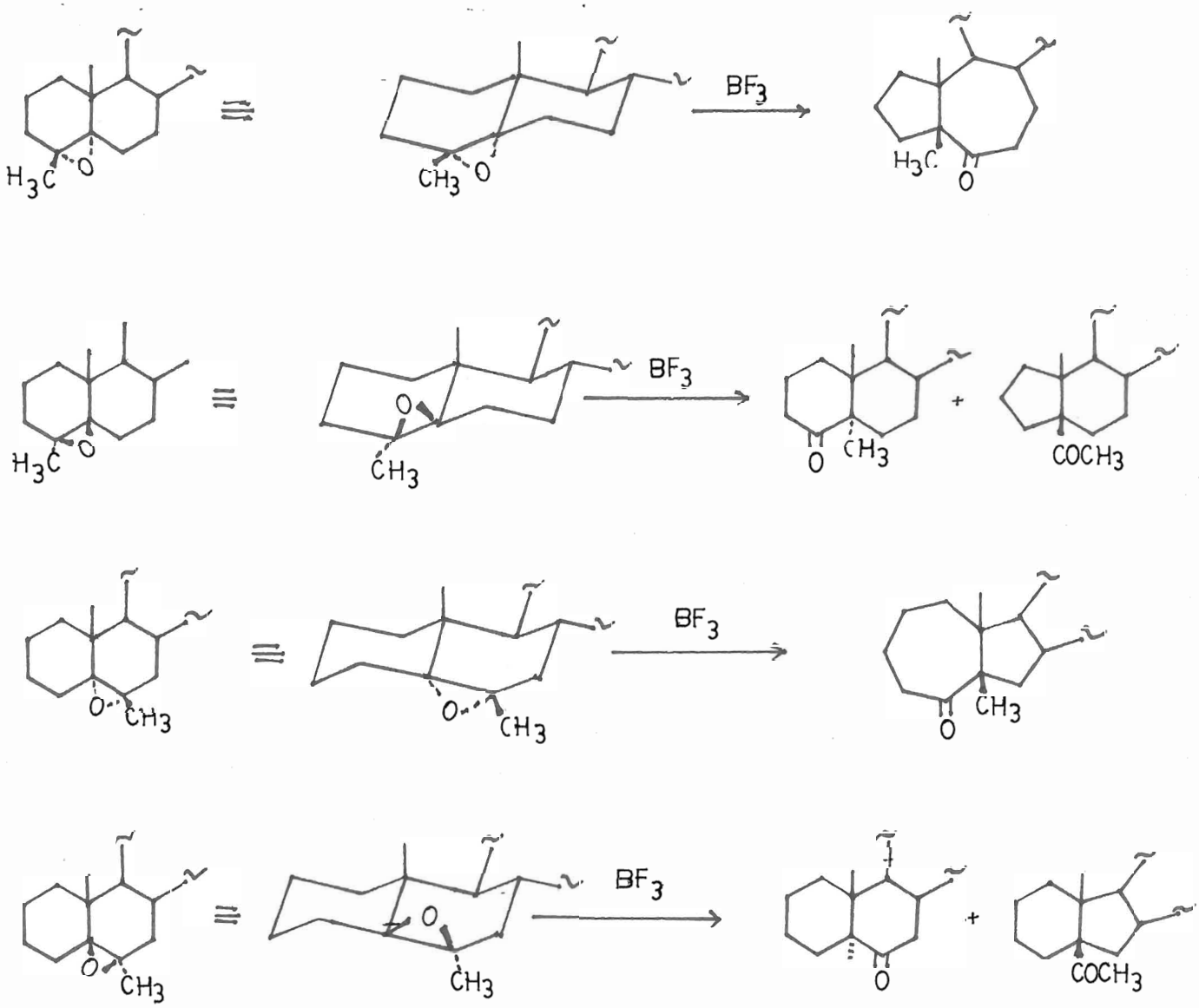


Figure 11

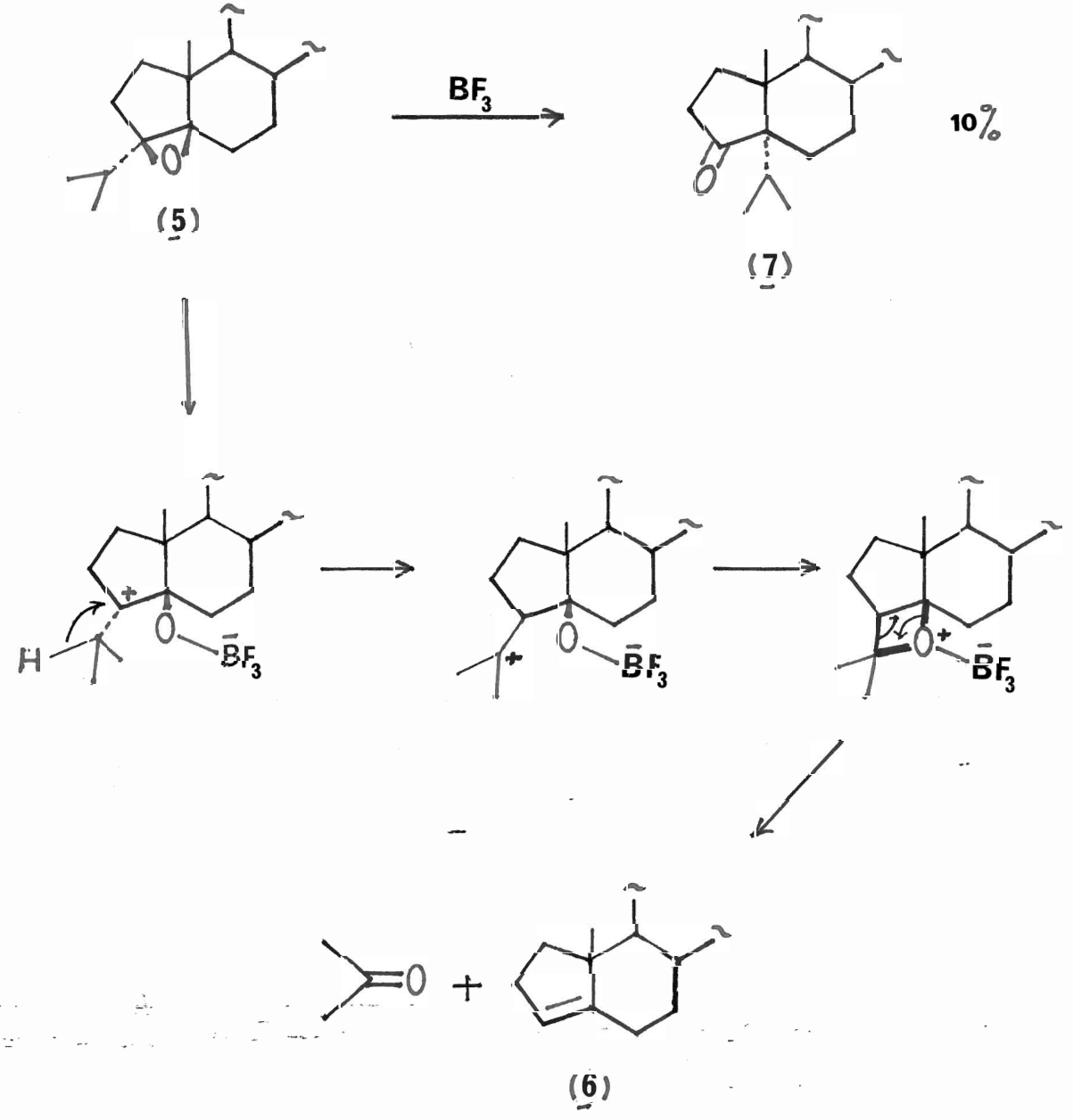


Figure 12

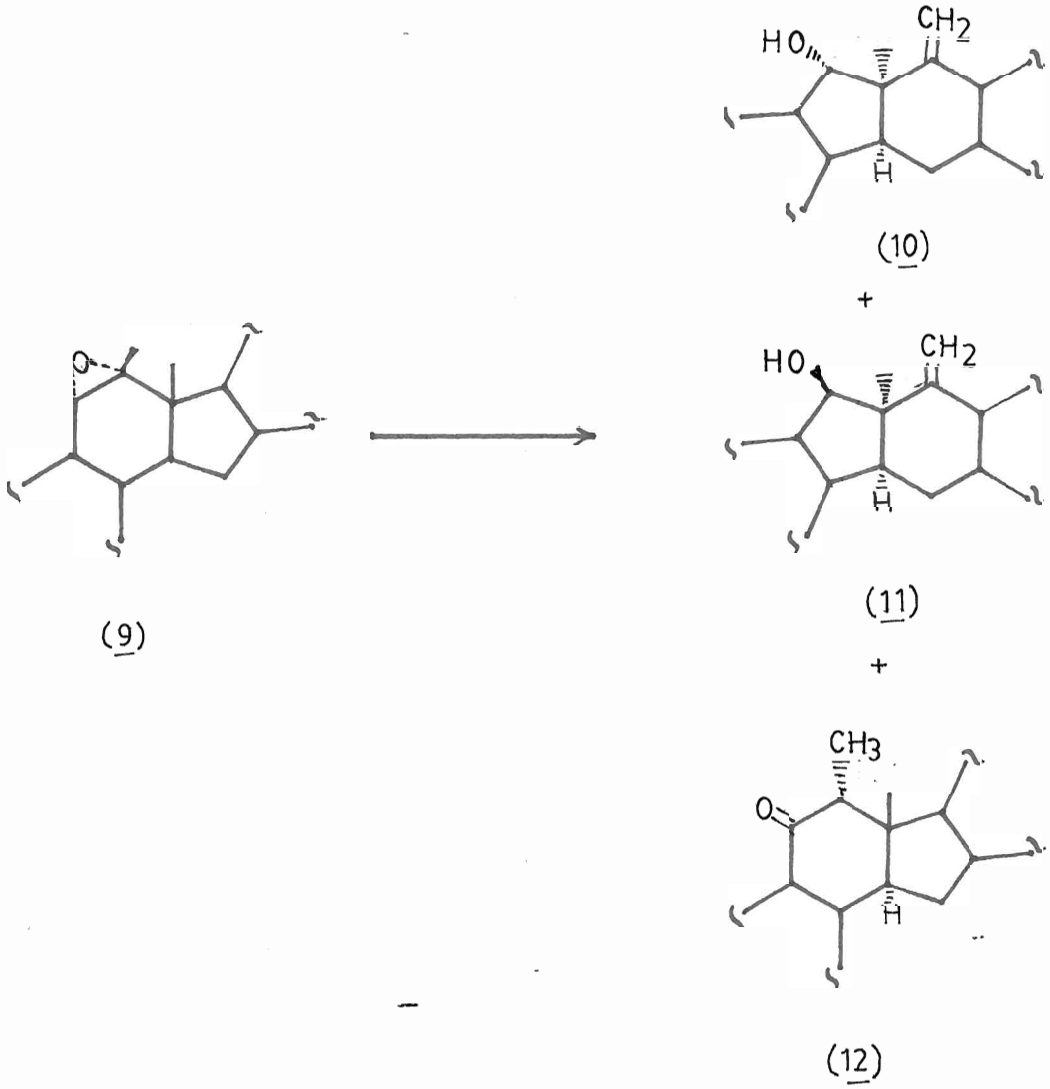


Figure 13

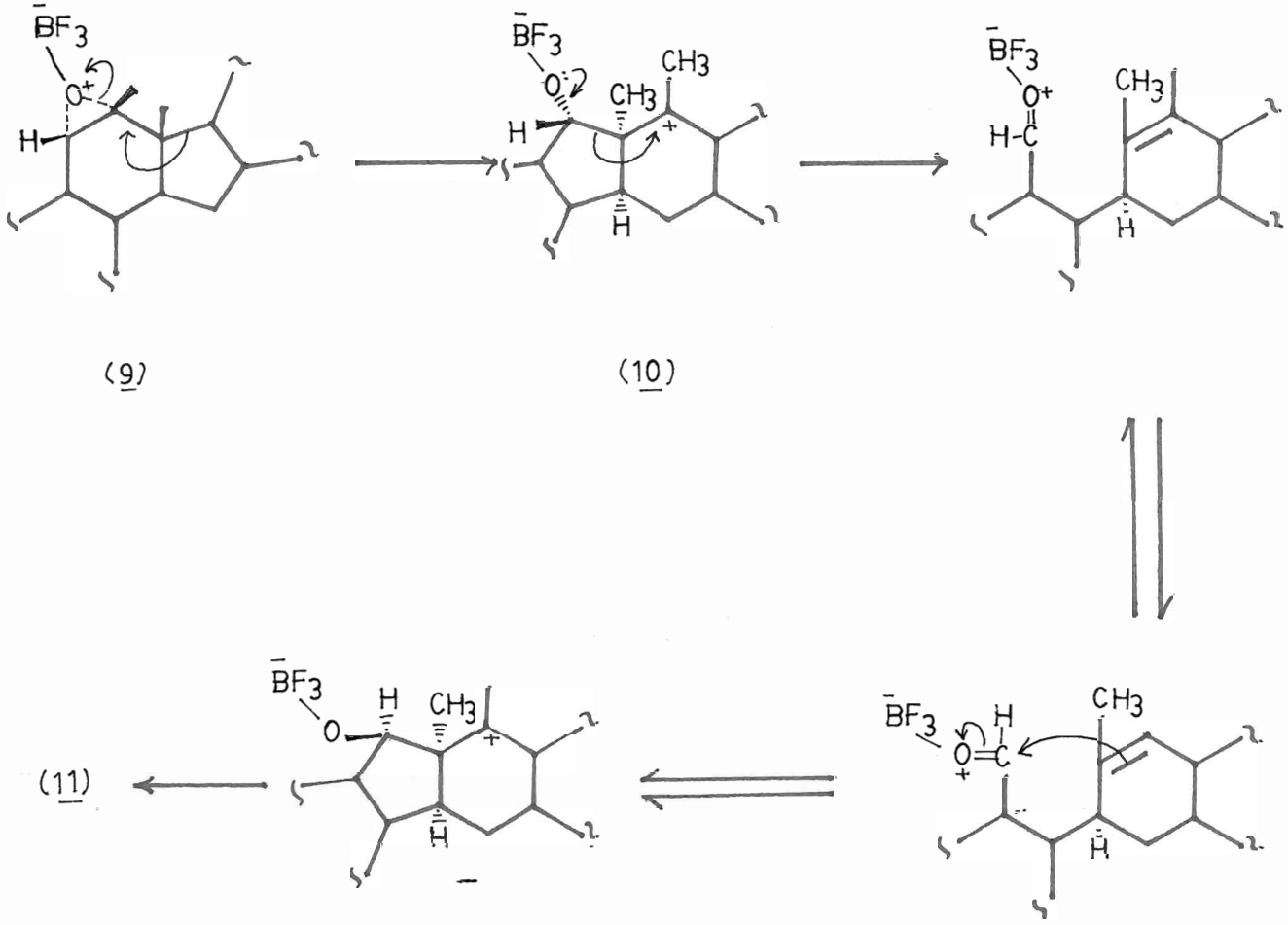
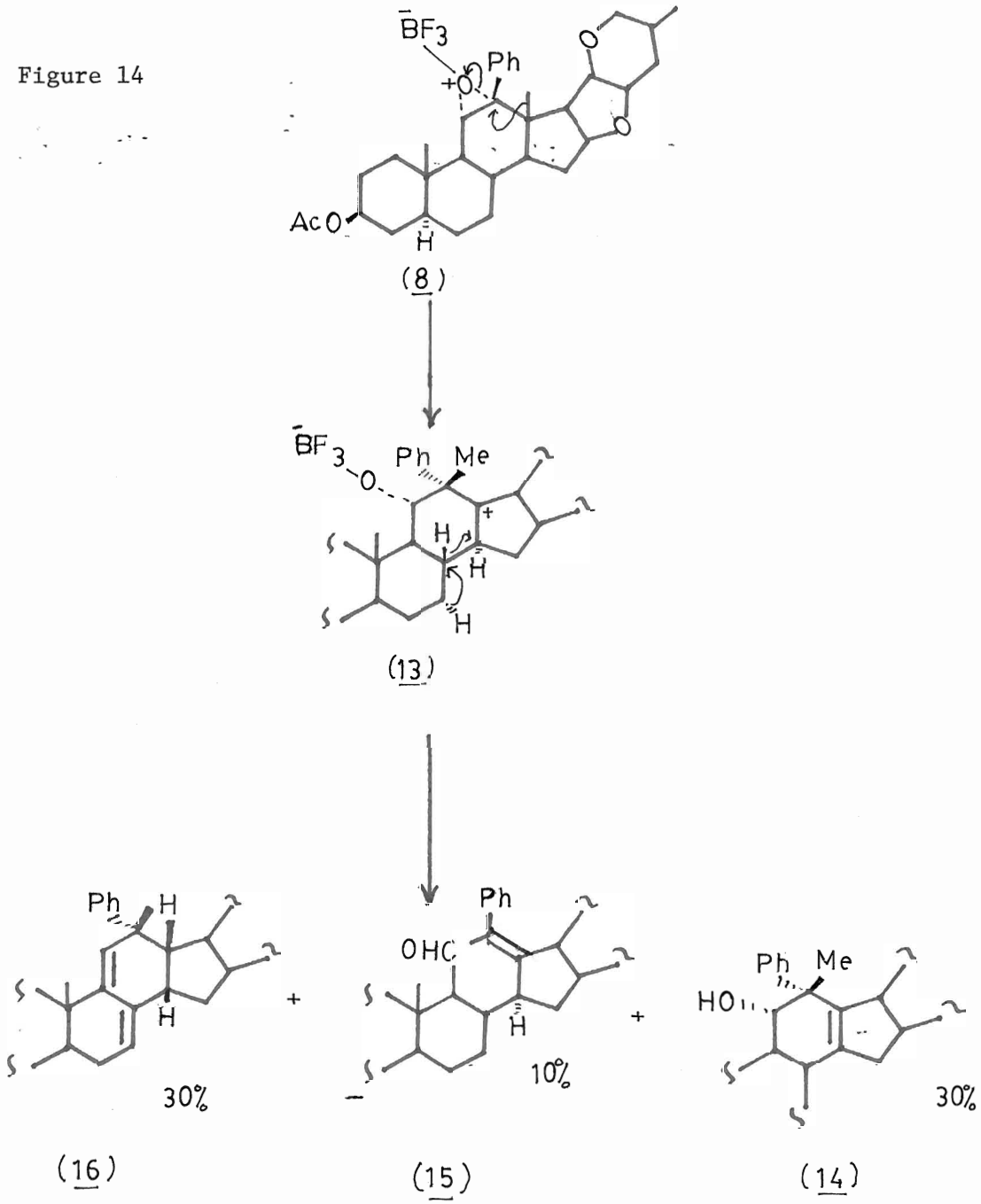


Figure 14


BF3
Ph
AcO
H
(8)
BF3
O
Ph
Me
H
H
H
(13)
Ph
H
H
30%
(16)
OHC
Ph
H
10%
(15)
Ph
Me
HO
30%
(14)

Participation of neighbouring groups during epoxide openings have been studied in great detail.[28-36] Mastalerz and Morand[31] observed an interesting participation of the 19-hydroxy and 19-acetoxy groups in BF_3 catalyzed opening of 5,6-epoxides. Some examples of the participation of 19-hydroxyl in epoxide openings have been outlined in Figure 15 and 16. The reactions of epoxides (17) and (18) with BF_3 yielding corresponding products have been mechanistically represented in Figure 17.

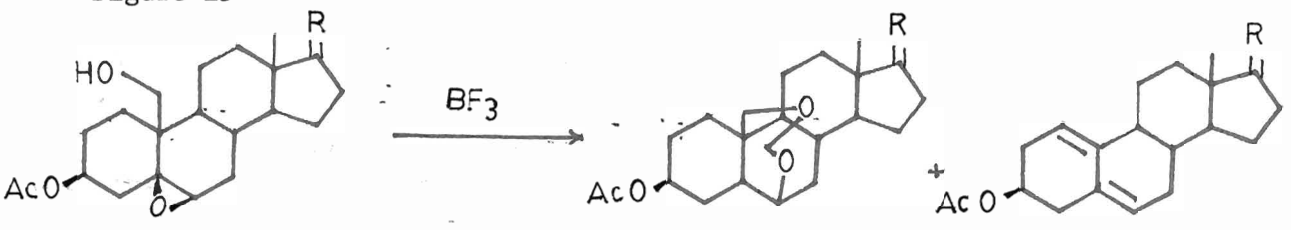
The epoxide ring openings using $LiAlH_4$ and sodium borohydride also have been investigated in some detail.[37-39] Nucleophilic opening of the epoxide ring in 4α,5α-epoxycholestane-7α-ol (27) with azide anion to give the product (28) has been reported[40,41] to derive an assistance from intramolecular association with the hydroxyl group.

The nucleophilic opening of 4α,5α-epoxy-17,17-cycloethylenedioxy-androstan-3β-ol (29) with propargyl magnesium bromide[42] gave an entirely different product (30), when the epoxide had 3α-ol (31) (Fig. 18). The 3β-oxygen coordinated with magnesium in the transition state (Fig. 19) and directed the attack at C-4 from top side leading to the formation of allene derivative (30).

However, the base promoted reactions of epoxides have not enjoyed thorough investigation. The reactions of epoxides with strong bases can occur by at least three major pathways, viz., rearrangement to allylic alcohols, to ketones, or by direct nucleophilic substitution.

Barton et al.[42] reported the base catalyzed isomerization of typical steroidal βγ epoxy ketones to the corresponding γ-hydroxy ketones (Fig. 20) and no neighbouring hydroxyl group participation was observed.

Figure 15



(17) R = H, C_8H_{17}

(18) R = O

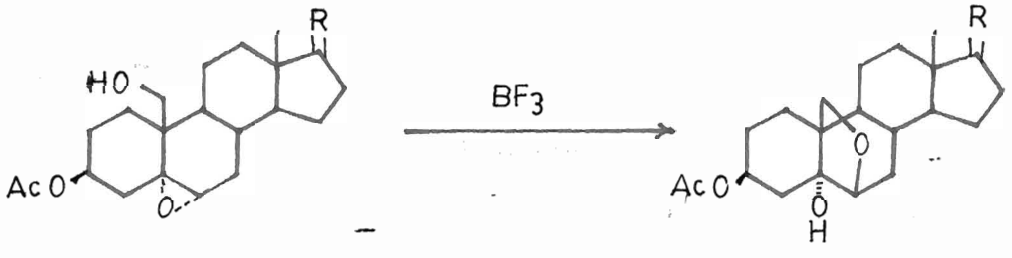
(19) R = H, C_8H_{17}

(21) R = O

(20) R = H, C_8H_{17}

(22) R = O

Figure 16



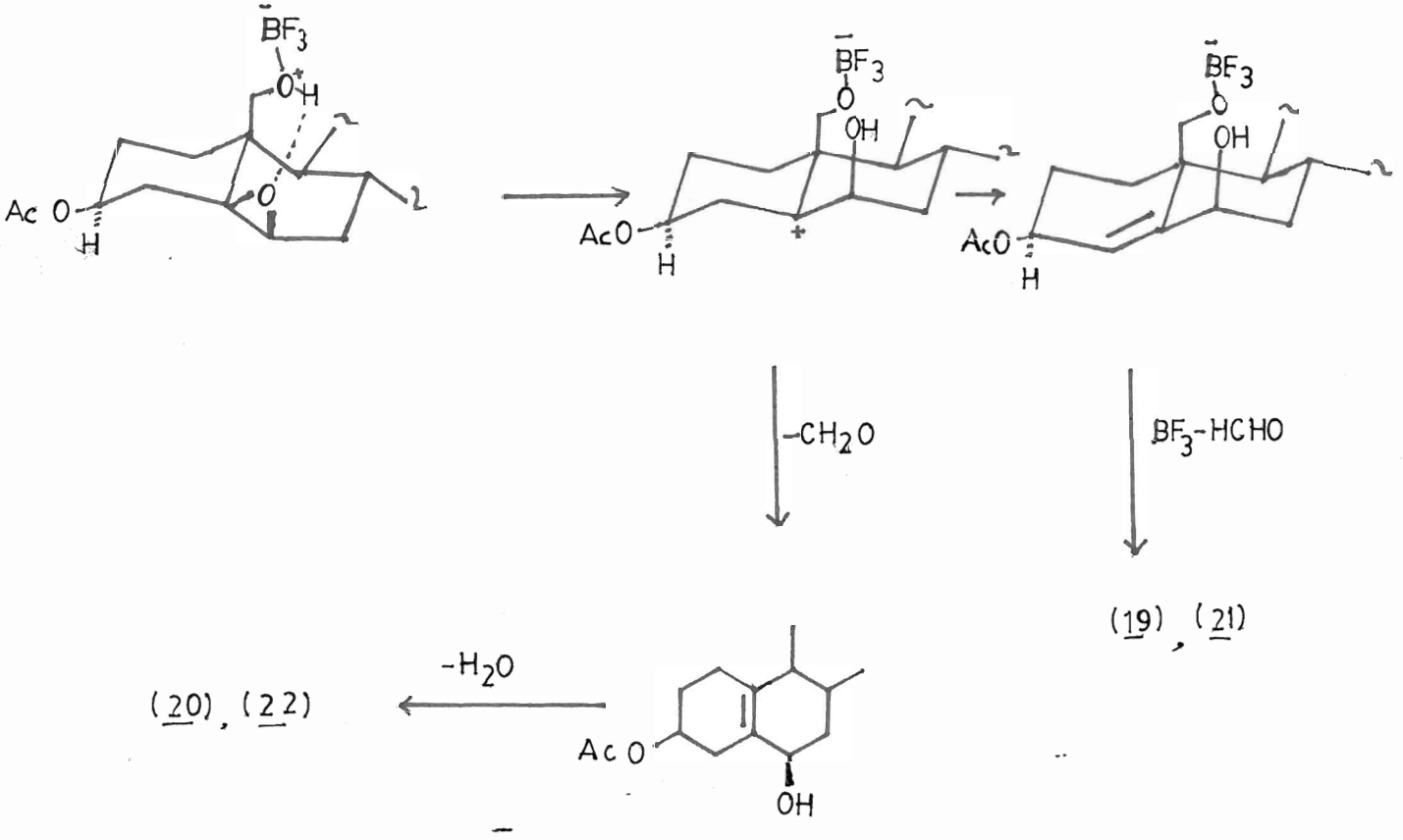
(23) R = H, C_8H_{17}

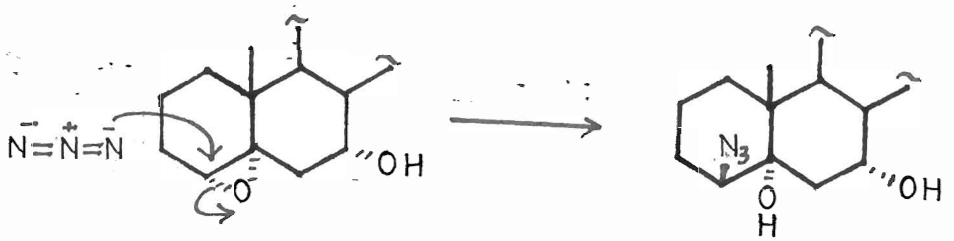
(24) R = O

(25) R = H, C_8H_{17}

(26) R = O

Figure 17


BF3
O
H
Ac O
H
O
BF3
O
OH
AcO
H
BF3
O
OH
AcO
H
-CH2O
BF3-HCHO
(19), (21)
-H2O
(20), (22)
Ac O
OH



(27) (28)

Figure 18

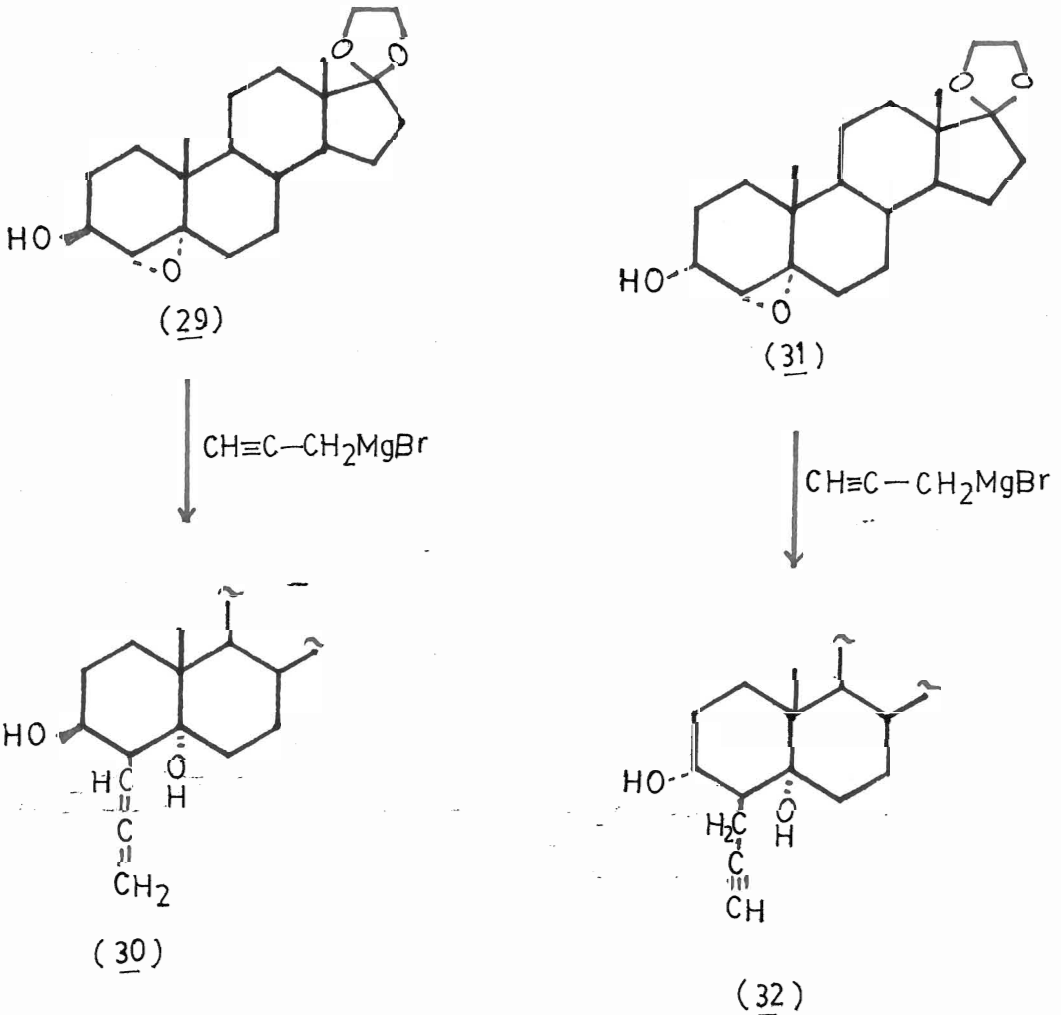
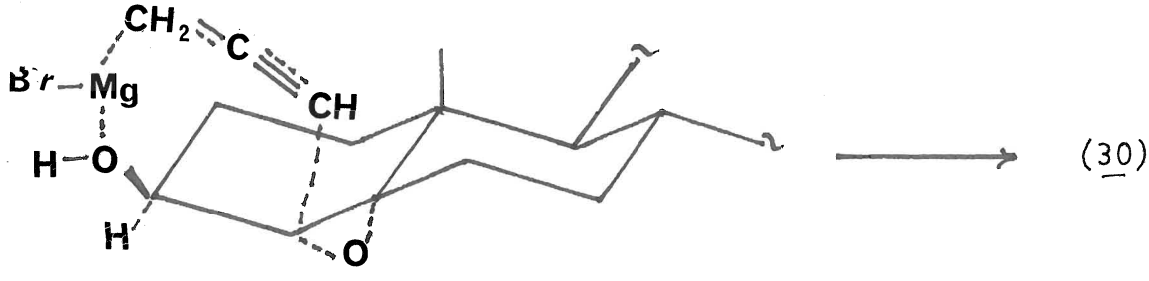
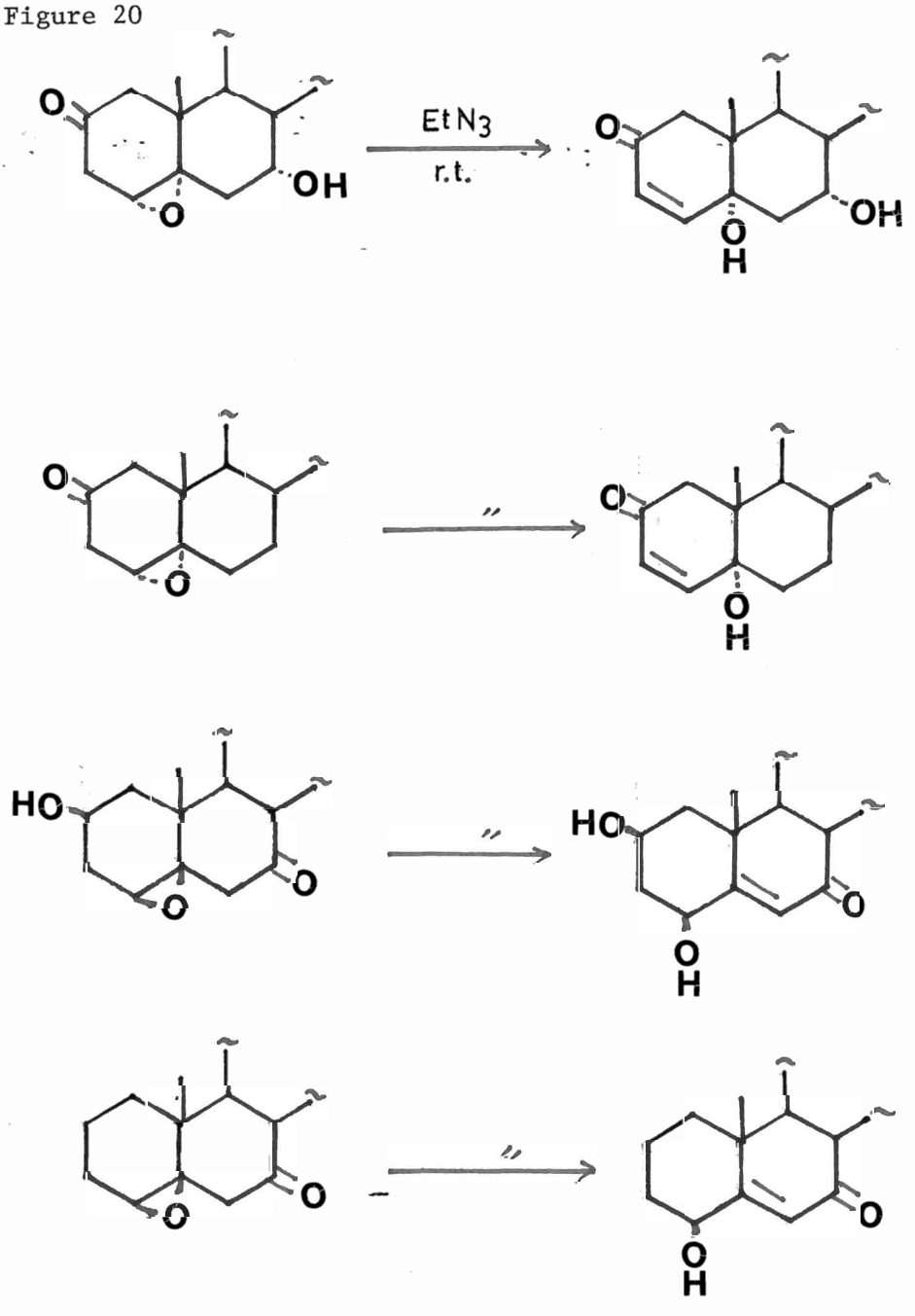


Figure 19



(30)

Figure 20

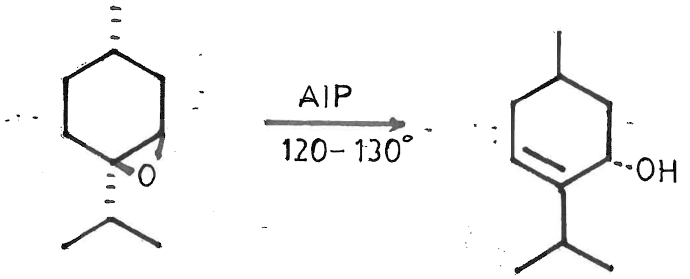


Aluminum isopropoxide (AIP) has been reported[43] to rearrange various epoxides to their corresponding allylic alcohols. It takes place in the presence of catalytic amounts of aluminum isopropoxide which leads to cleavage of the ether linkage at the site of the more substituted α-carbon of the oxirane ring. The stereoselectivity of the AIP rearrangement of 3,4-epoxy-p-menthones, has disclosed that the cis isomer (33) reacts rapidly at 120-130°C to yield almost exclusinvely the predicted[44] trans-3-p-menthen-5α-ol (34). Under identical conditions, 3,4-epoxy-trans-p-menthane (35) do not react but afforded allylic alcohol (36) as the major product at slightly elevated temperature.

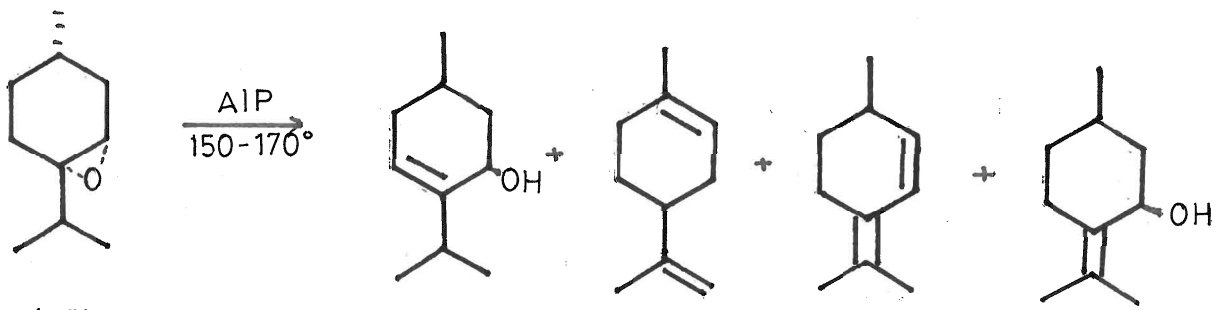
The 6,7-epoxydihydrocitronellol[45] (37) yielded the corresponding glycol (38), α-cedrene epoxide (39) was rearranged to 9-α-hydroxy-8(15)-cedrene[46] (40) and β-cedrene epoxide (41) afforded 15-hydroxy-8-cedrene[47] (42).

Similarly, 3,7-dimethyl-6,7-epoxy-1-octene (43) gave 3,7-dimethyl-1,7-octadiene-6-ol[48] (44).

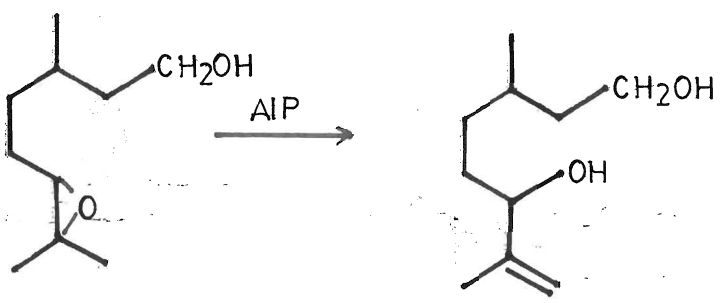
The AIP epoxide rearrangement, exemplified in (43), involves an electrophilic co-ordination of the oxirane oxygen and results in the cleavage of its link with the tertiary α-carbon, in a mechanism which may proceed through a six-membered transition state (43a), involving a proton transfer from the less substituted carbon in β-position of the oxirane oxygen (Fig. 21). This results in the formation of isopropanol and the mixed alcolate (45). This mixed alcolate proceeds to attack another molecule of epoxide (43), thereby liberating a molecule of allylic alcohol (44) and restoring (45) to continue the chain reaction.



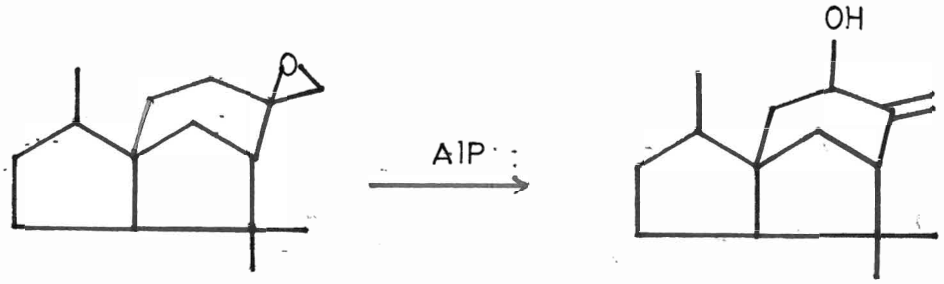
(33) (34)



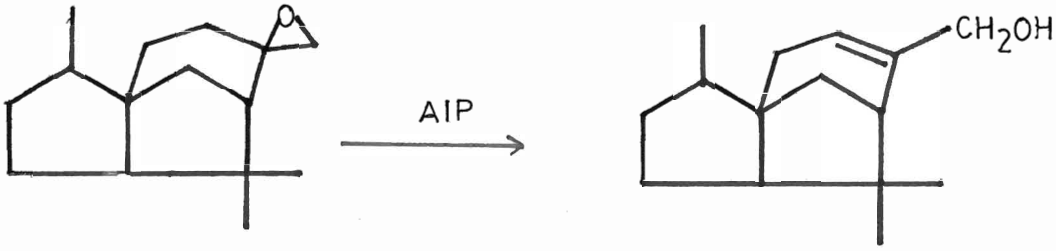
(35) (36)



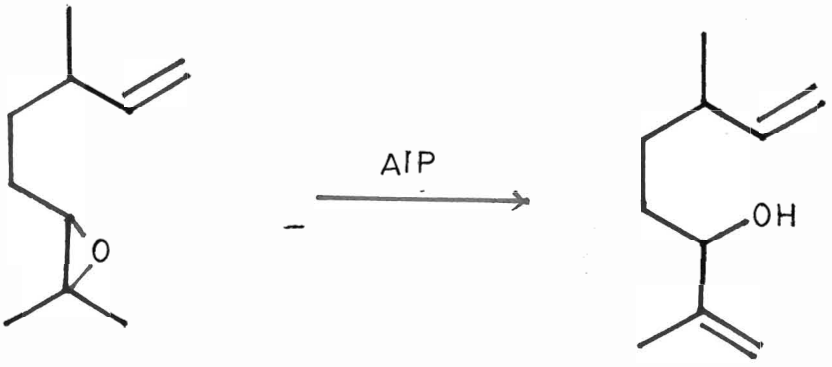
(37) (38)



(39) (40)

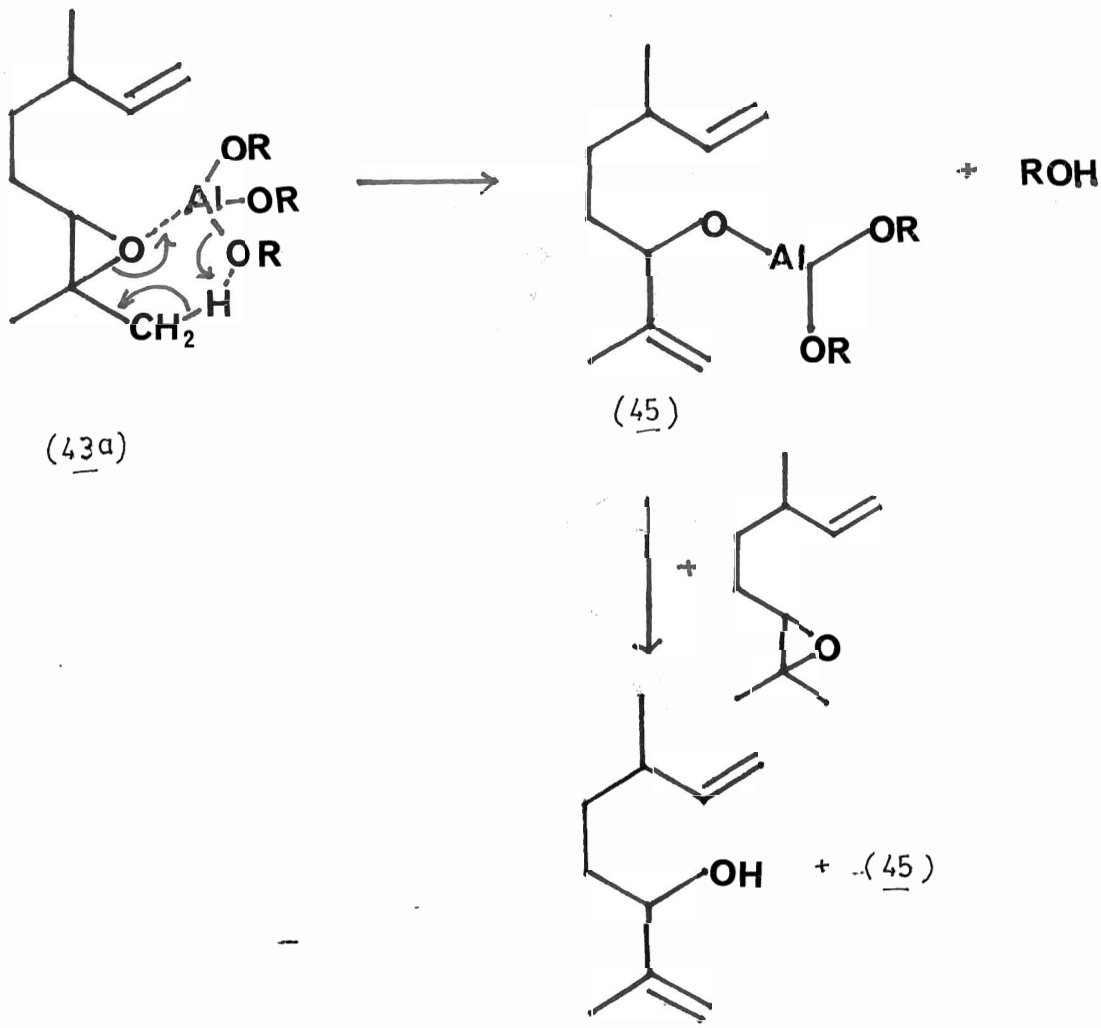


(41) (42)



(43) (44)

Figure 21



(43a)

(45)

(44)

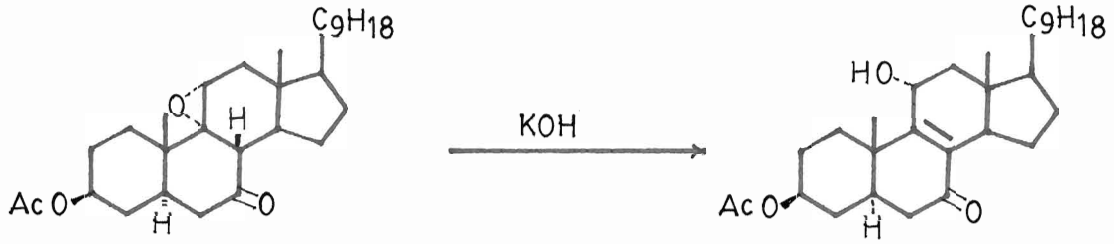
Grigor et al.[49] reported the isomerization of β,γ-epoxy ketones to the corresponding allylic alcohols in refluxing potassium hydroxide solution. The 9,11α-epoxide (46) rearranged by trans elimination to the corresponding 11α-allylic alcohol (47), while the 7,8α-epoxide (48) seemed to rearrange by cis elimination in two ways (Fig. 22).

Exploration of epoxide chemistry continued but the attention was diverted towards the use of poorly nucleophilic, strong organic bases. During the polymerization studies of substituted ethylene epoxides, Price and Carmelite[50] observed that the reaction of tetramethylethylene epoxide (51) with potassium t-butoxide in DMSO proceeded solely by attack at methyl producing α,α,β-trimethylallyl alcohol (52) in 98% yield. The trimethyl ethylene epoxide (53) similarly afforded two isomeric alcohols, namely, α,α-dimethylallyl alcohol (54) (80%) and α,β-dimethylallyl alcohol (55) (15%).

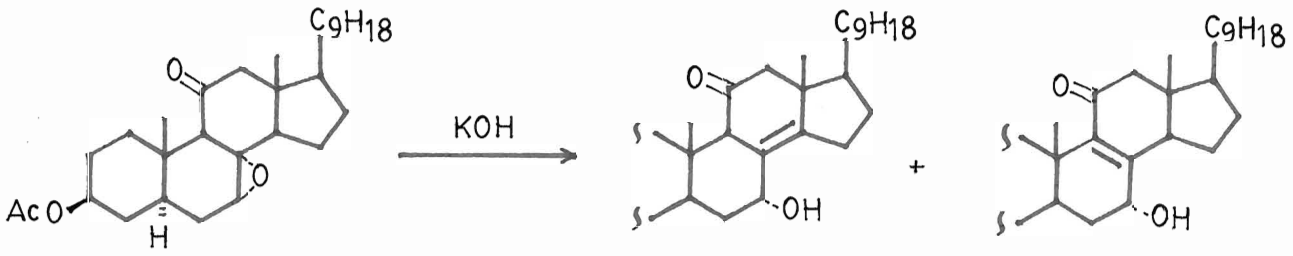
The reactions of aryl substituted epoxides (56) with potassium t-butoxide in t-butanol resulted in the formation of corresponding allylic alcohols (57) via β-proton abstraction to give a carbanion which in turn gave the unsaturated alcohols (Fig. 23).

In recent years, an additional dimension has been added to epoxide chemistry through the utilization of more strong bases, alkyl lithiums and lithium alkylamides. These reagents have been extensively utilized and have provided better preparative synthetic routes in many organic syntheses.[52] For instance, the reaction of α-epoxide (58) with sec-butyl lithium gives exclusively oxetane (59), while the corresponding β-epoxide (60), under similar conditions fragmented to produce allylic alcohol[53] (61).

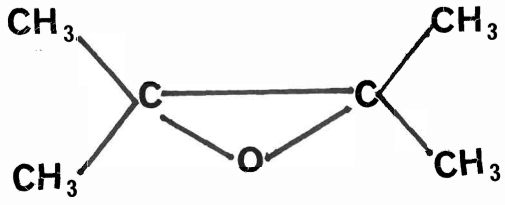
Figure 22



(46) (47)



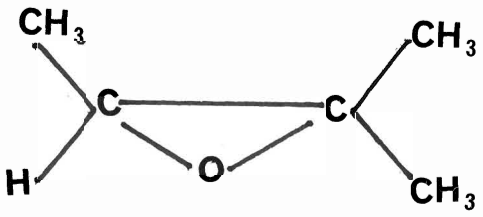
(48) (49) (50)



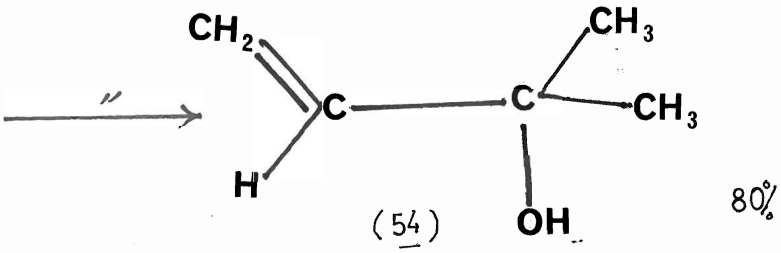
(51)

(52)

98%



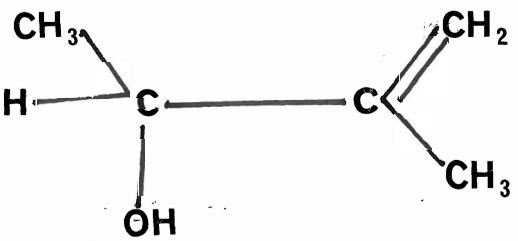
(53)



(54)

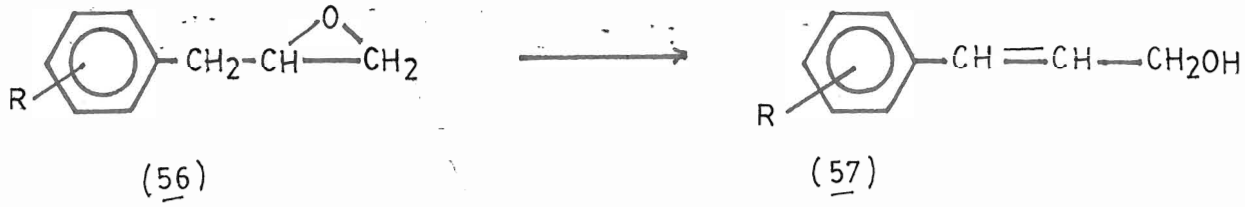
80%

+



(55)

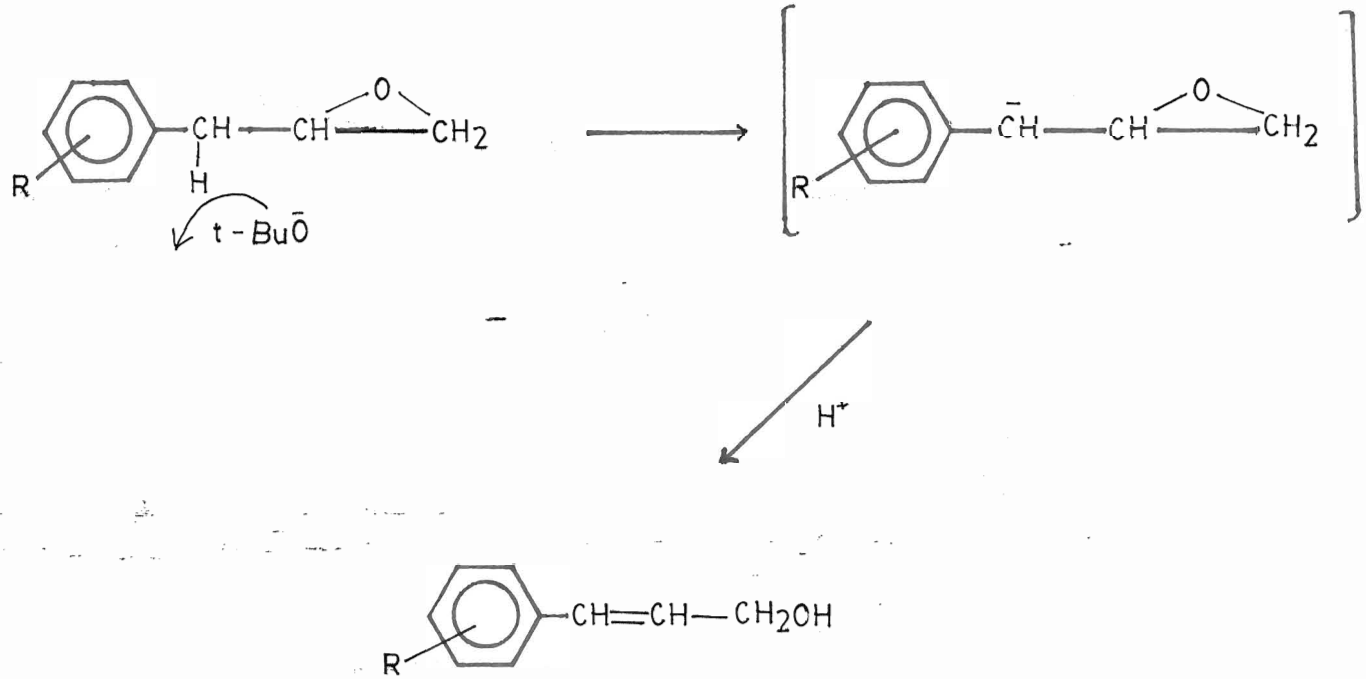
15%



(56) (57)

R = H, p-Cl, p-F, p-Me, p-Me_3C, p-MeO, m-Cl, m-MeO

Figure 23

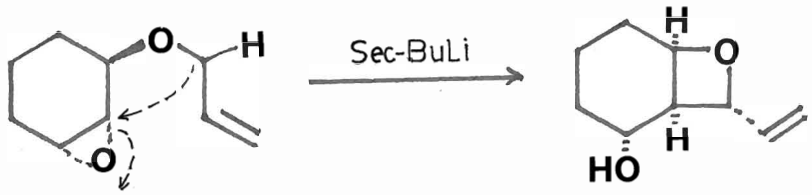


(57)

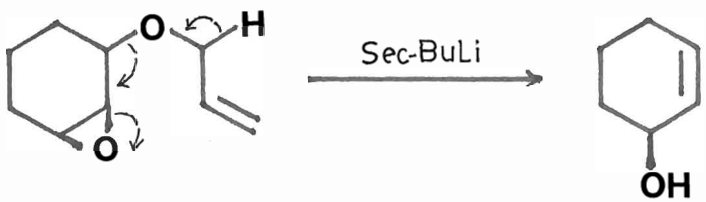
Y. Goani[59] made use of n-butyllithium in the conversion of epoxy sulfones (62) into their corresponding cyclopropyl sulfones (63) (Fig. 24). The same reaction was extended in the syntheses of highly strained molecules, bicyclobutanones and spirobicyclobutanones. The reaction of epoxides[54] (64) with n-butyllithium gave bicyclobutyl sulfones (65) in varying yields depending upon the nature of the substituents (Fig. 25).

Crandall and Lin[55] reported that t-butylethylene epoxide (66) under reflux conditions reacted with n-butyllithium giving trans-di-t-butyl ethylene (67) in 64% yield along with small amounts of nucleophilic adduct, 2,2,5,5-tetramethyl-3-hexanol (68) (6%). Similar results were obtained[55] obtained using n-butyllithium. A carbenoid mechanism was suggested because when the alcohol (68) was added to the reaction mixture, it did not undergo dehydration to produce (67). The metalation, therefore, seemed to occur at the α-carbon of oxirane ring, producing an organolithium intermediate (69). This species (69) can undergo α-elimination to give a carbene, followed by the insertion into the carbon lithium bonds of a second t-butyllithium molecule to form (70). This unusual chemical entity was proposed[55] to eliminate spontaneously the elements of lithium oxide, thereby generating the olefinic bond (Fig. 26).

Another example of carbenoid insertion came when the trans-di-t-butyl-ethylene epoxide (71) on treatment with t-butyllithium generated three products. Since in this system β-elimination is also possible, the formation of diastereomeric cyclopropyl alcohols (72) and (73) can be rationalized by the sequence of metallation, α-elimination, and carbene

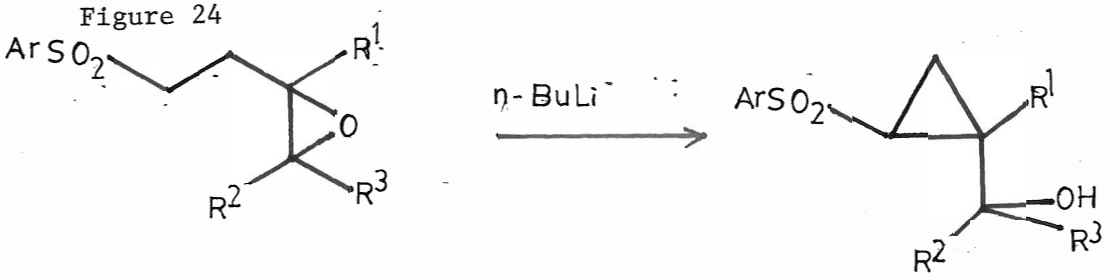


(58) (59)



(60) (61)

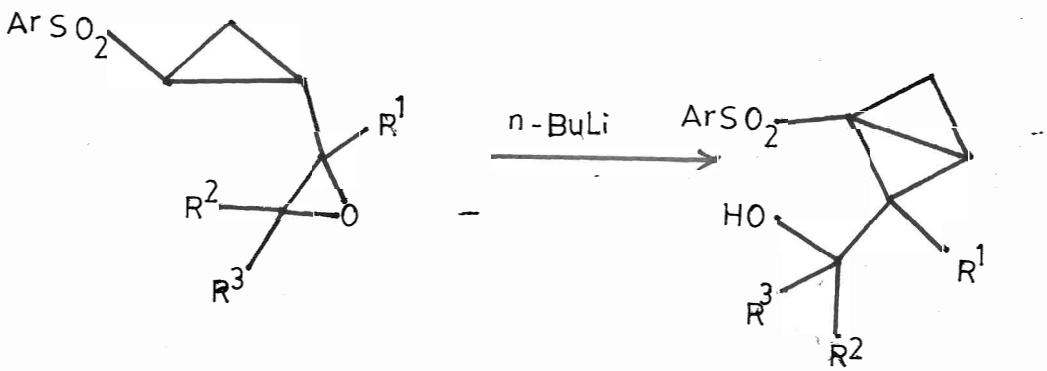
Figure 24



(62) (63)

a , R^1=Me, R^2=R^3= H ; b, R^1=H , R^2=R^3=Me ; c , R^1=H , R^2=R^3=Me3

Figure 25



(64) (65)

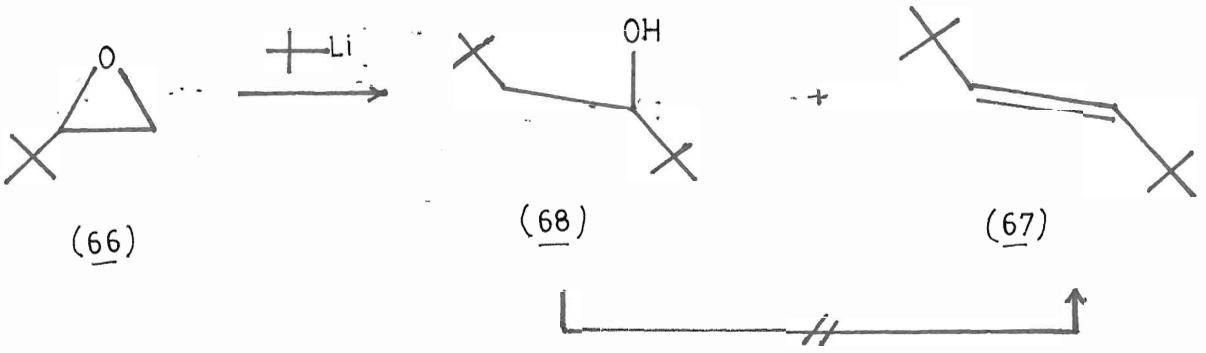
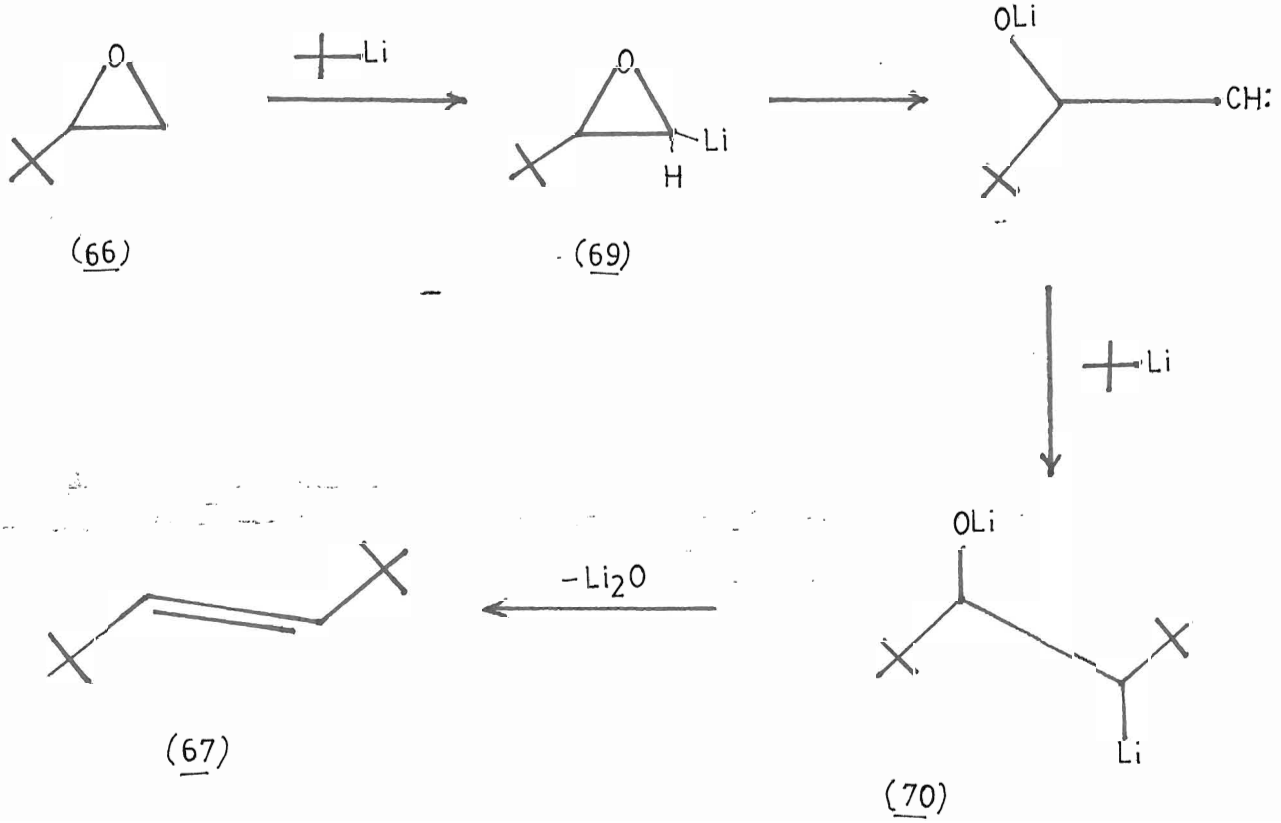


Figure 26



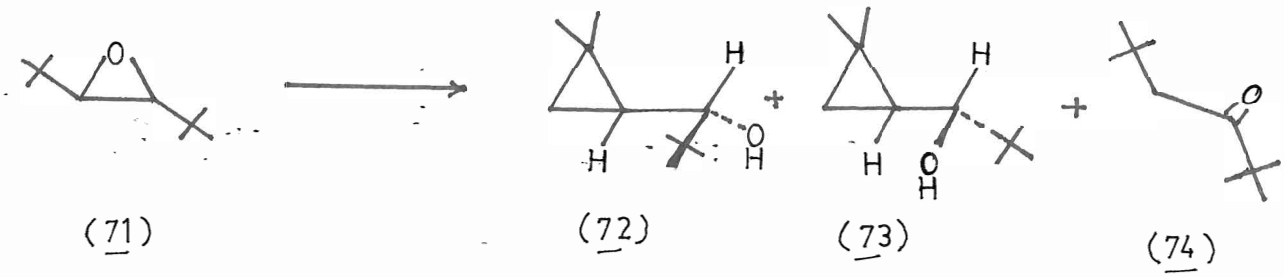
insertion into a C-H bond of the adjacent t-butyl substituent (Fig. 27). The ketone (74) most probably arises by an α-elimination mechanism involving a 1,2-hydrogen shift from a carbene intermediate (Fig. 27).

The carbenoid intermediate mechanism in the case of α-elimination was supported when trans-2-bicyclo-[3.1.0]-hexanol (78), though a minor product, was obtained when 5,6-epoxy-1-hexene (75) was reacted with t-butyllithium. Its formation appears to require carbenoid addition to the neighbouring double bond.

Nozaki et al.[59] reported the isomerization of both trans- and cis-epoxydodecane to trans-2-cyclododecenol on treatment with n-butyllithium. The allylic alcohol was formed by the β-elimination from either of the epoxides (Fig. 28).

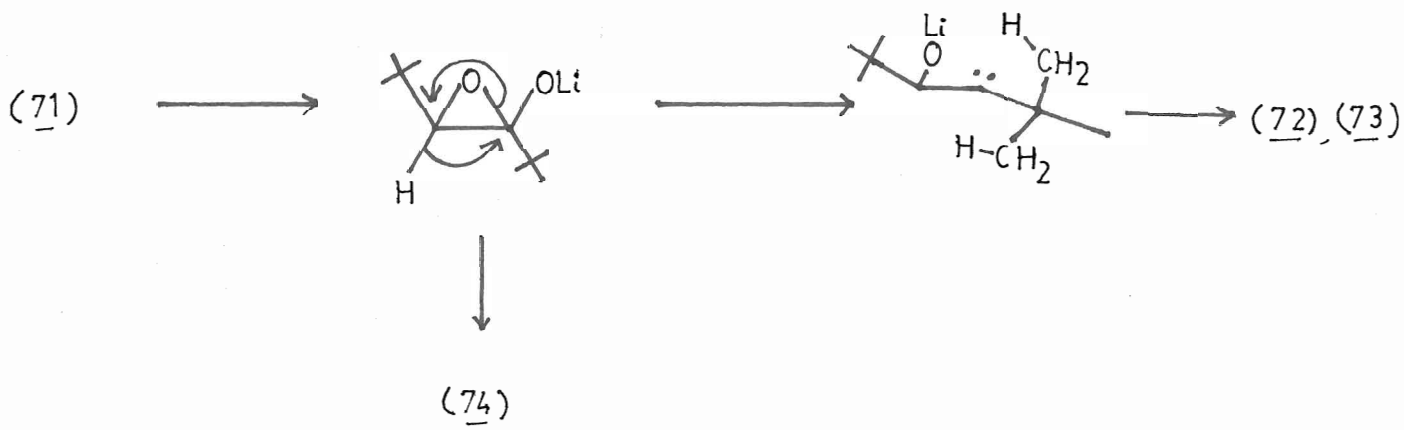
The reaction of an epoxide with a strong base such as lithium diethyl amide may take a number of courses depending on the structure of the oxirane. Cope and Heeren[60] have shown that with both cis- and trans-4-octene oxide, the elimination occurs via abstraction of a proton from a carbon adjacent to the epoxide ring (β-hydrogen abstraction). Labelling studies showed that both the cis- and trans-epoxides gave the same allylic alcohol (trans olefin), to the exclusion of the cis-isomer (Fig. 29).

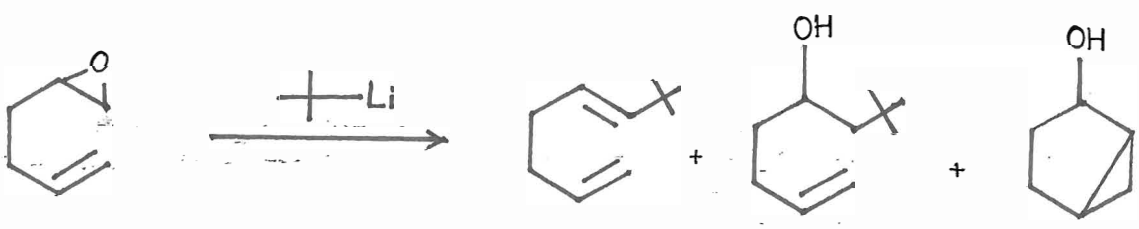
During the reaction of a series of phenyl substituted ethylene epoxides with lithium diethylamide, Cope et al.[61] observed that cis-stilbene oxide was isomerized to deoxybenzoin, trans-stilbene oxide gave diphenyl acetaldehyde, triphenylethylene oxide produced benzhydrylphenyl ketone, and 1,1-diphenyl-2-p-tolylethylene oxide yielded benzhydryl-p-tolylketone (Fig. 30). Tetraphenylethylene oxide was found inert under similar conditions.



(71) (72) (73) (74)

Figure 27





(75) (76) (77) (78)

Figure 28

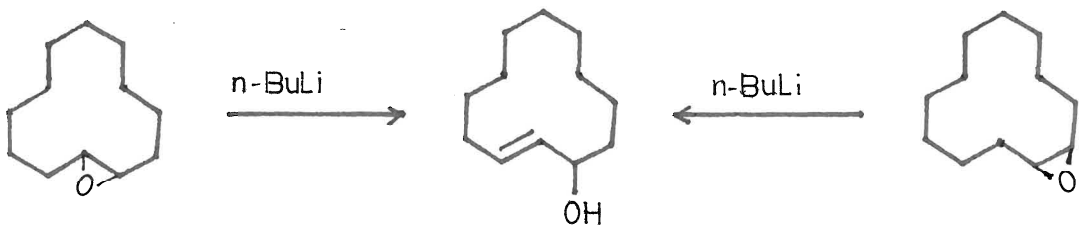


Figure 29

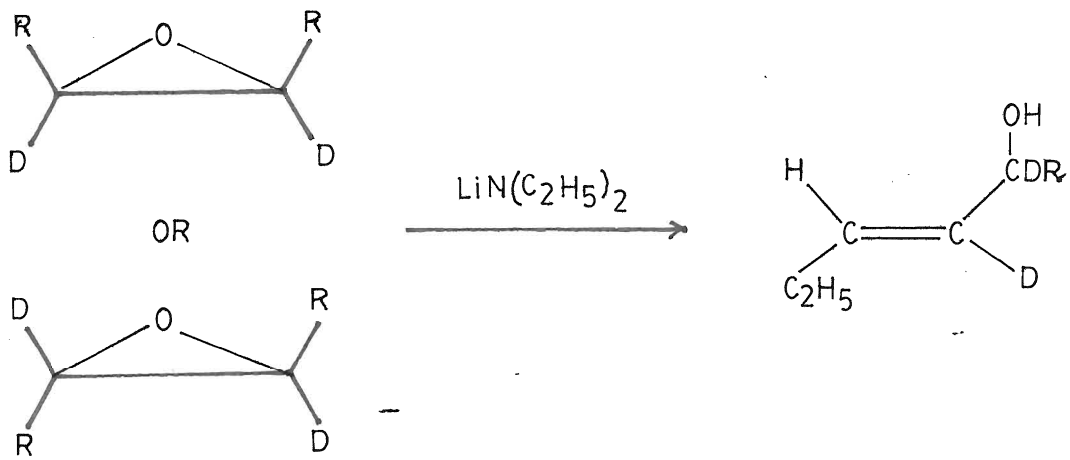
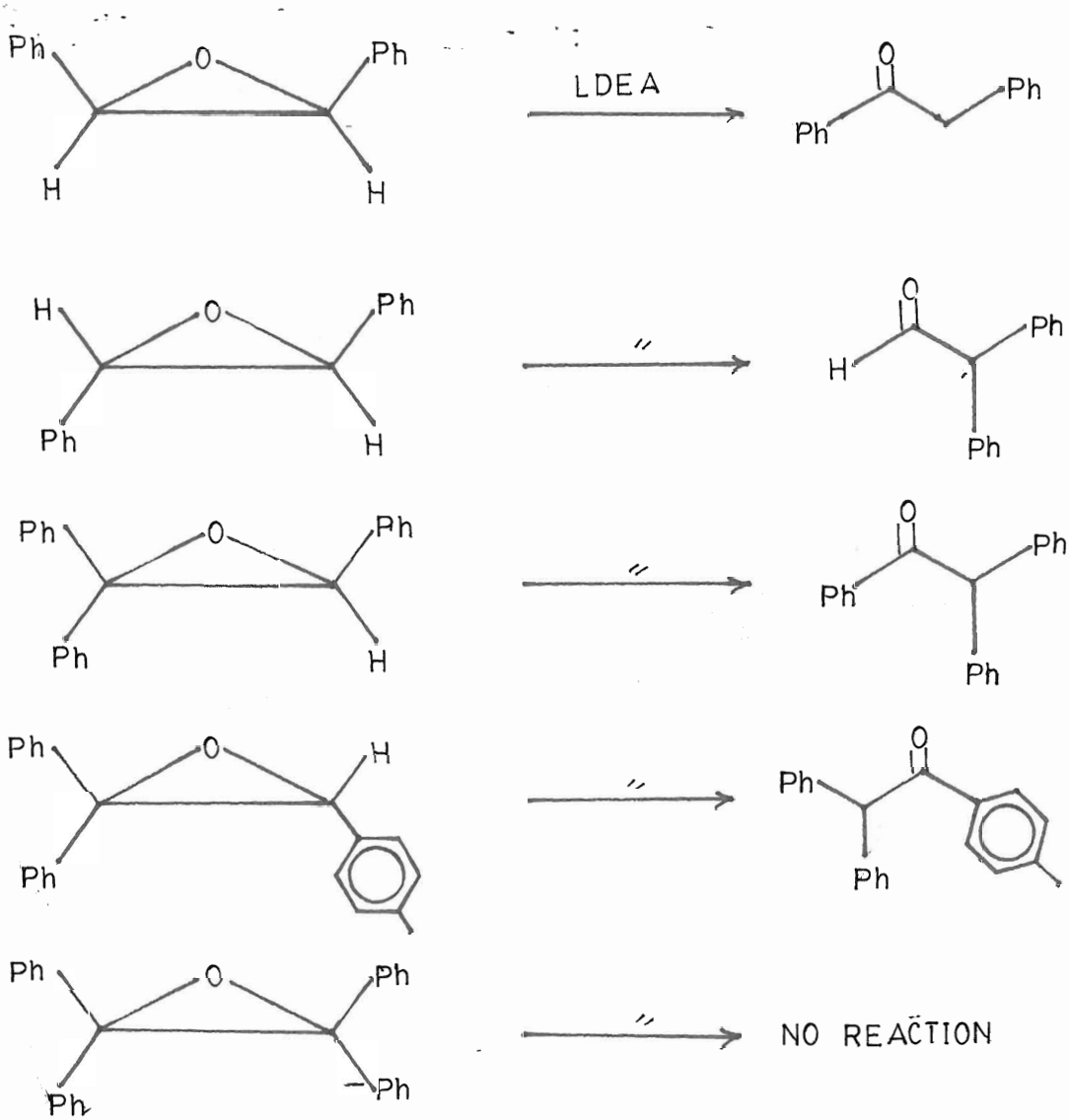


Figure 30


Ph
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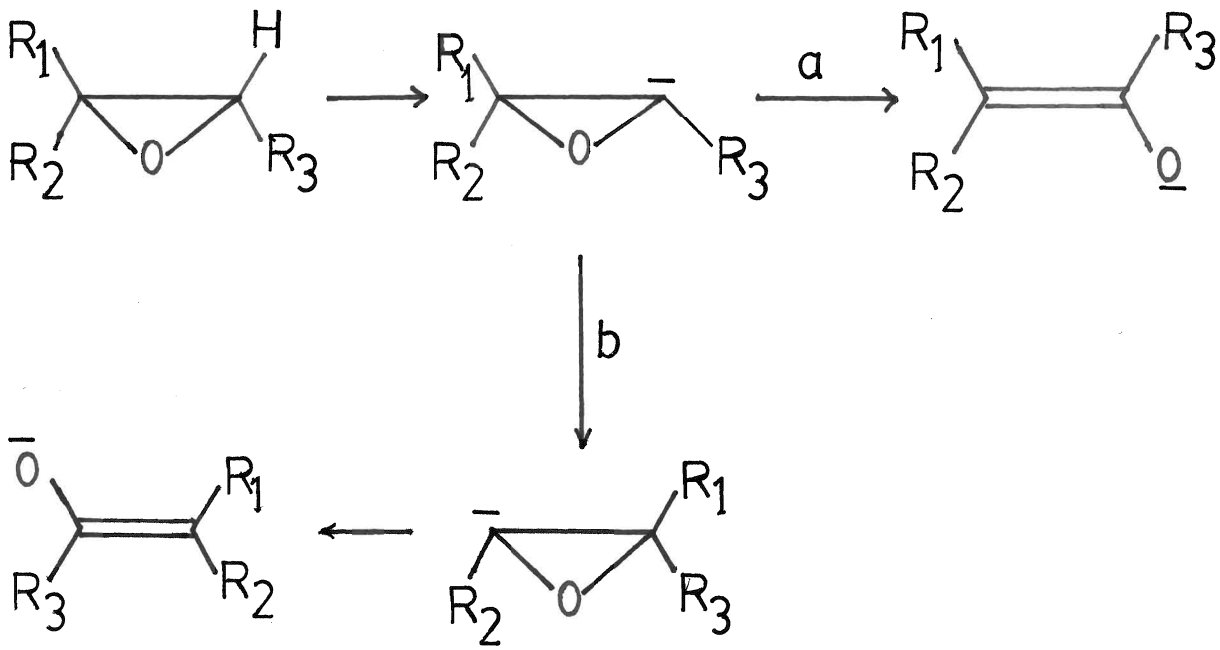
NO REACTION

These reactions proceed by removing hydrogen attached to the epoxide carbon (αH). Once the proton has been removed, the ring opening can occur directly to give the enolate anion of a carbonyl compounds (path a) or rearrangement might occur (path b) giving rise to an isomeric product (Fig. 31).

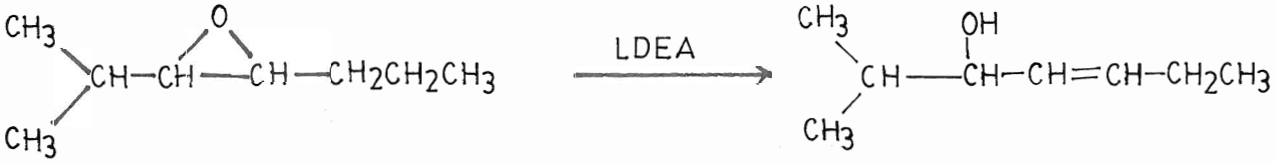
Very high selectivity has been observed[62] in reactions of unsymmetrical epoxides with lithium diethylamide, leading to allylic alcohols. The proton abstraction occurs most readily from the least substituted carbon in open chain systems, large factors separating primary from secondary, and, in turn, from tertiary proton abstraction. 2-Pentene oxide (79) yielded 1-pentene-3-ol (80) as the exclusive allylic alcohol and 2-methyl-3-heptene oxide (81) gave only 2-methyl-4-heptene-3-ol (82). Nearly statistical product distribution was observed from 2-methyl-2-butene oxide (83), which afforded 2-methyl-3-butene-2-ol (84) (41%) and 3-methyl-3-butene-2-ol (85) (59%).

Cope and Tiffany[63] were apparently the first workers to observe an unusual base catalyzed rearrangement of an epoxide when dealing with cyclooctatetraene mono-oxide. The results obtained with the medium-ring compounds are particularly interesting, because of trans-annular reactions. For example, the reaction of cis-cyclo-octene oxide (86) with lithium diethylamide results in the formation of mainly trans-annular product, endo-cis-bicyclo-[3,4,0]-octan-2-ol (87), while trans-cyclo-octene oxide (88) yields exo-cis-bicyclo-[3,4,0]-octan-2-ol (89) and cycloheptane carboxyaldehyde (90) as the major products[64] (Fig. 32). Similar reactions have been also observed with cis- and trans-cyclodecene oxides[65] (Fig. 33).

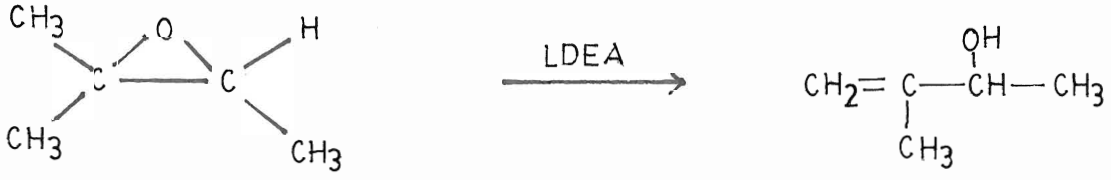
Figure 31



(79) (80)

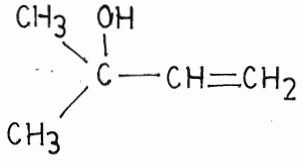


(81) (82)



(83) (85)

+



(84)

Figure 32

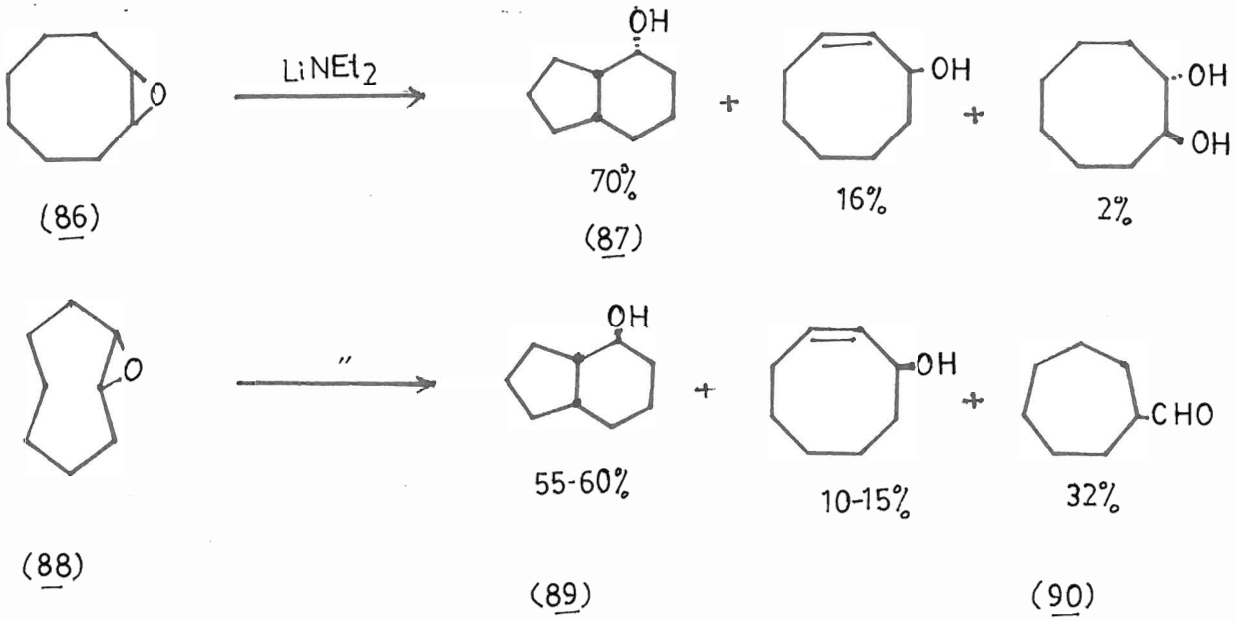
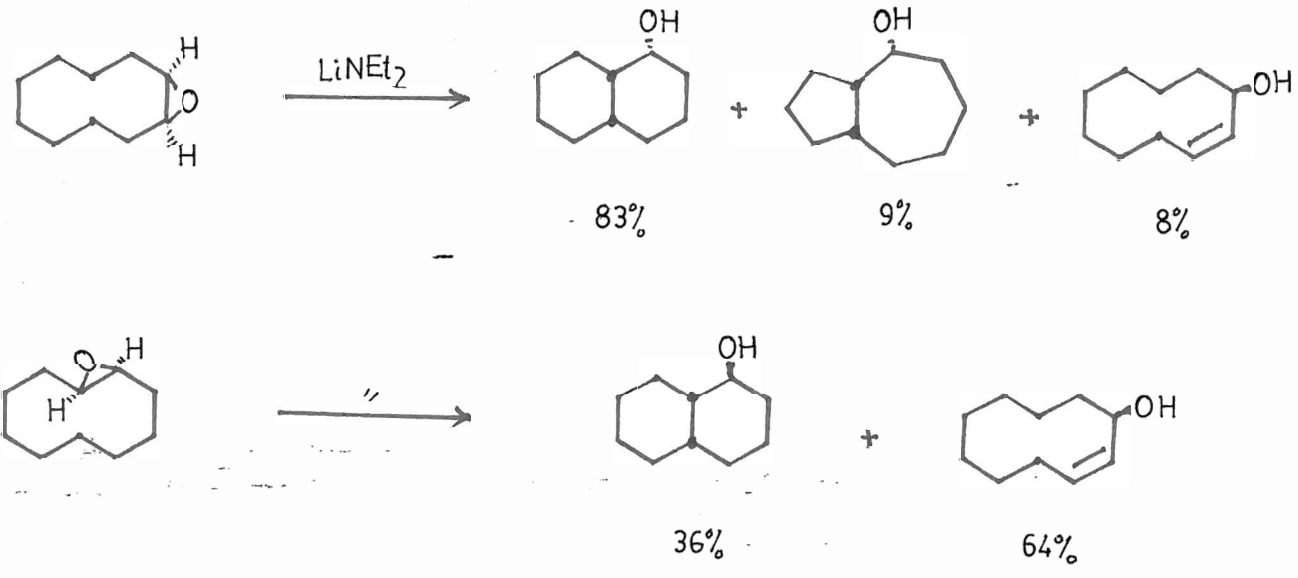


Figure 33



Two mechanisms have been proposed for the formation of bicyclic products. These mechanisms are presented as concerted, since the products are single stereoisomers, for example, cis-cyclodecene oxide produced endo-cis-bicyclo[5,3,0]-decan-2-ol (Fig. 34A). The paths a and b have been distinguished[66] by deuterium tracer studies with deuterated epoxide (91).

The concerted carbene mechanism, path b, was supported because the bicyclic products formed from the deuterated epoxide (91) retained only one deuterium (Fig. 34B).

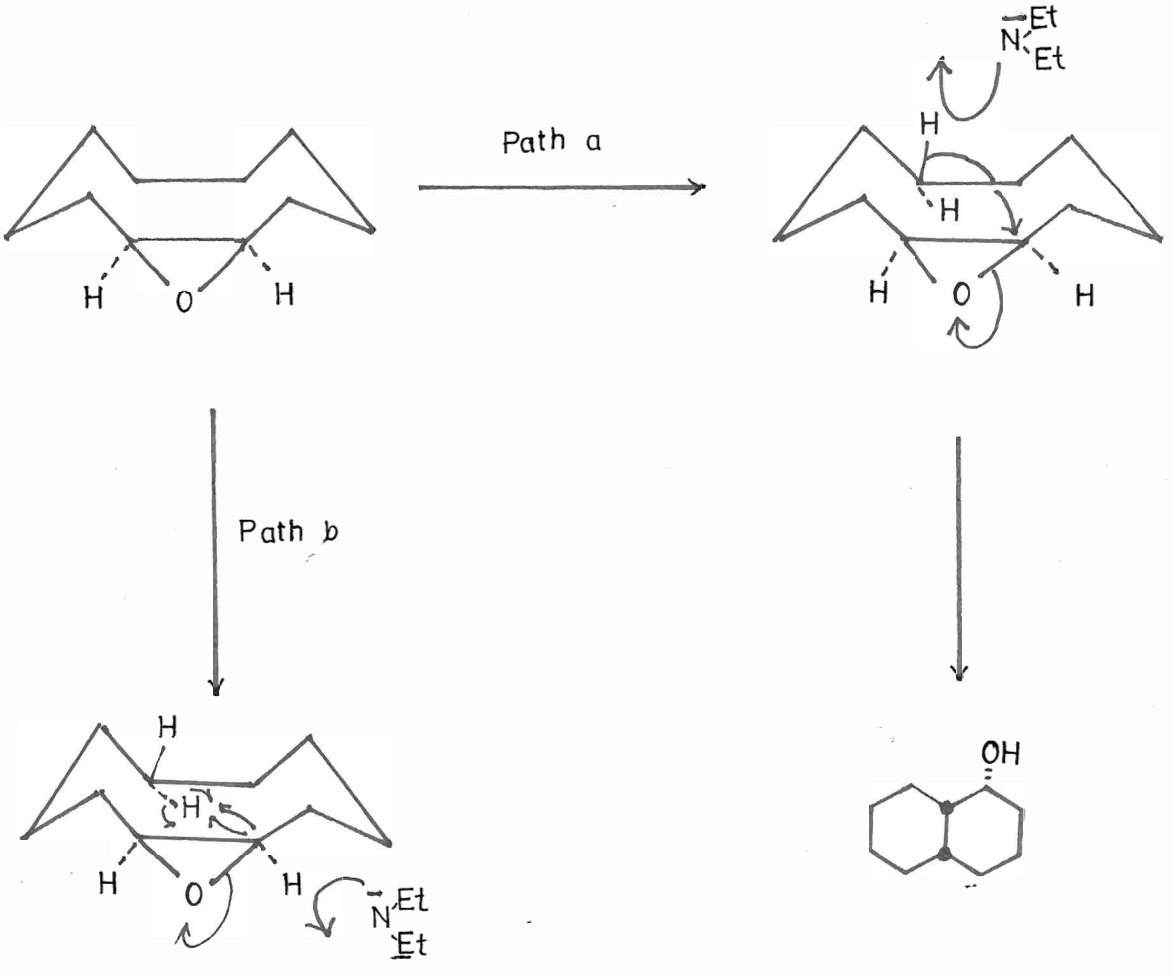
Further support for a concerted carbene intermediate was obtained from the reaction of deuterated cis-cyclo-octene oxide[66] (92).

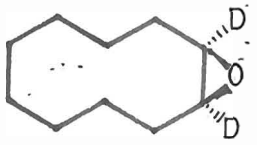
Fascinated by transannular reactions, Crandall and Lin[67] observed only two isomeric allylic alcohols as major products in the β-elimination from 1-t-butylcyclo-octene oxide (93). No transannular product was detected from the reaction mixture. This deviation from normal transannular reaction was probably due to destabilization created by t-butyl group in the transition state which suppressed α-elimination (Fig. 35).

The interest in base-promoted rearrangements of medium ring epoxides leading to some transannular products continued. To investigate the predicted carbene insertions,[68,69] 3,4-epoxycyclo-octene (96) was treated with lithium diethylamide and gave cis-bicyclo-[3,3,0]-oct-7-ene-endo-2-ol (97) as the major product along with 3-cyclo-octenone (98). The 5,6-epoxy-cyclo-octene (99) afforded[68] a mixture of 3,5- and 2,4-cyclo-octadienol as initial products to (98).

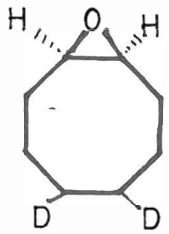
Niether of the above epoxides ((96), (99)) showed any evidence for carbenoid reaction involving addition to intramolecular double bond.

Figure 34A



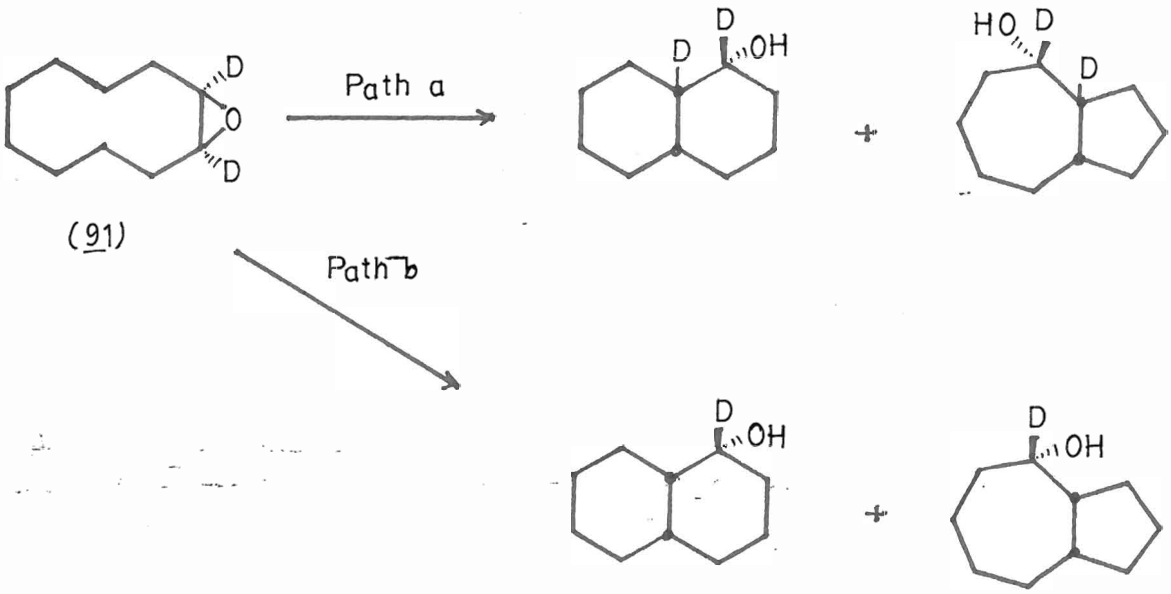


(91)



(92)

Figure 34B



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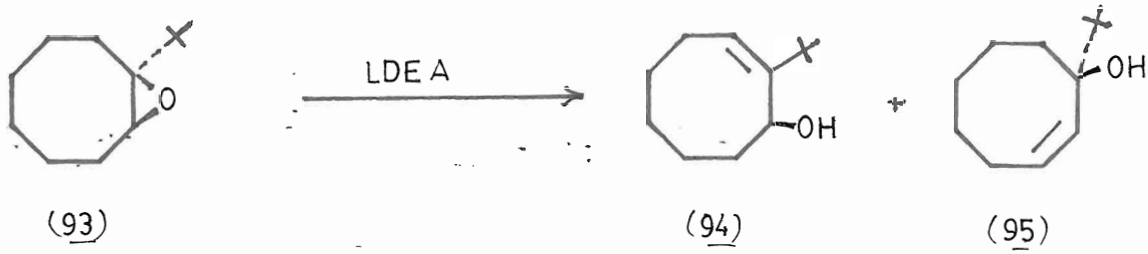
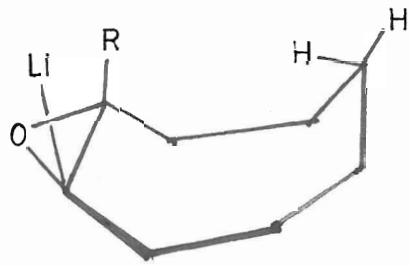
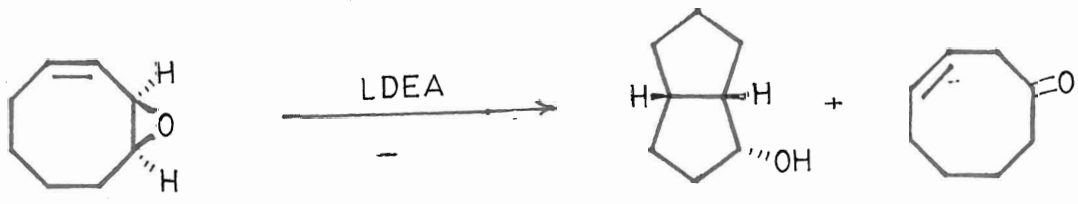


(93) (94) (95)

Figure 35



R = t-Butyl



(96) (97) (98)

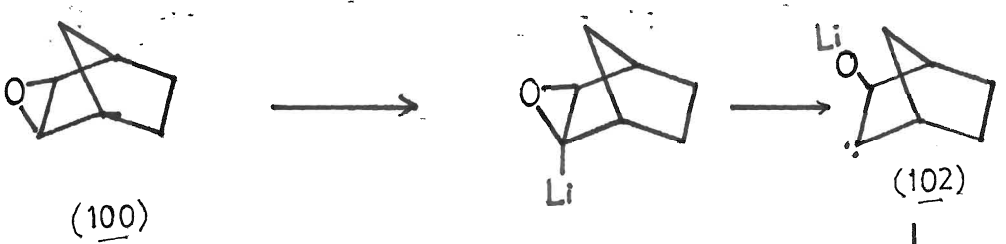
(99)

In connection with carbenoid reactions of epoxides, on further investigations, Crandall et al.[57,70] reported a smooth base promoted isomerization of exo-2,3-epoxybicyclo-[2,2,1]-heptane (100) to nortricyclanol[71] (101). The proposed mechanistic pathway for this transformation involved carbene (102) or its carbenoid equivalent as a key intermediate.

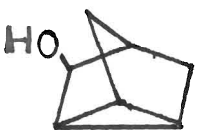
By deuterium labelling methods, it has been proved that reversible metalation occurs at the epoxide ring and base does not remove the exo hydrogen of the transannular bridge.

The endo-5-methyl derivative (103) was transformed into the analogous tricyclic alcohol (104), whereas epoxide (105), with both the transannular endo-positions blocked with methyl groups, isomerises to the bicyclic ketones[72] (106). These observations further supported a carbenoid mechanism for the transannular rearrangements.

The β-elimination in base induced epoxide rearrangements is always preferred when the molecule provides the required conformation for β-elimination. For example, 2-pinene oxide (107) was clearly rearranged[57] with the allylic alcohol (108). Here the ring methyl group can accommodate the required atomic arrangement for β-elimination without being unduly subjected to the steric shielding effect of the gem-dimethyl bridge. When this ring methyl group is replaced by hydrogen, the major products are ketonic, arising by α-elimination. For instance, apopinene oxide (109) produces ketones (110) and (111) dominating over allylic alcohol (112). In this case, the molecule can not assume the conformation suitable for β-elimination, which is further retarded by the steric effect of gem-dimethyl groups, and the transannular reaction does not occur because the

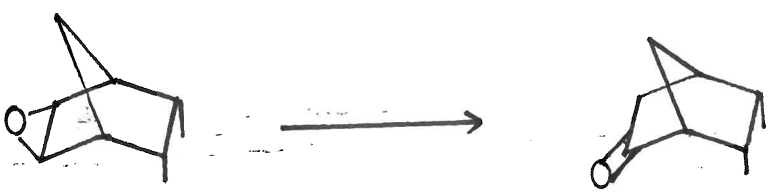


(100) (102)

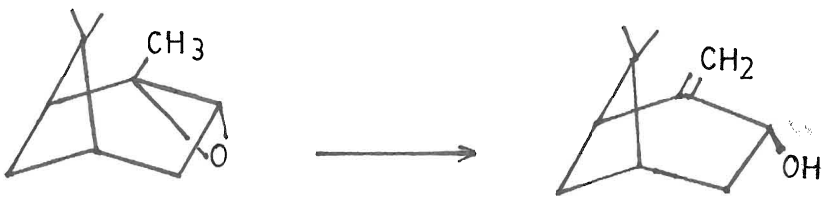


(101)

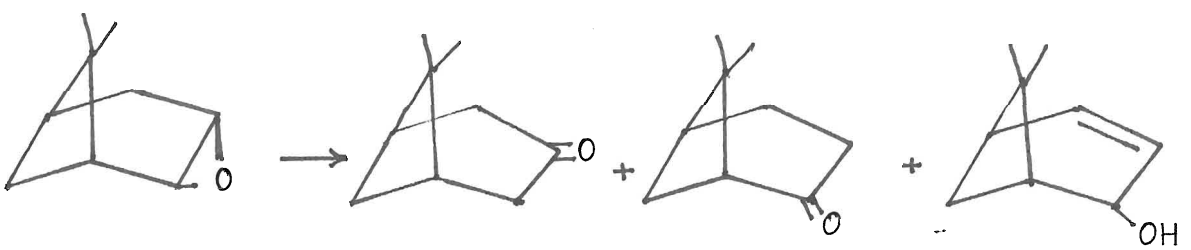
(103) (104)



(105) (106)



(107) (108)



(109) – (110) (111) (112)

opposing methylene group is not in close proximity.[57]

The lithium diethylamide induced rearrangement of epoxides to allylic alcohols is remarkable for its high selectivity, e.g., stereoselectivity (formation of trans-olefins in open chain systems)[60,62] and regioselectivity (exclusive, or nearly so, abstraction of proton from the least substituted carbon).[57,62,71,72]

The base-promoted rearrangements of a series of propylidene cycloalkane oxides[73] to corresponding allylic alcohols exhibit marked regioselectivity, with endo-cyclic olefinic product being formed preferentially, with the exception of propylidene cyclohexane oxide which gave exocyclic olefin (Fig. 36).

These observations support a syn elimination mechanism, with a very specific cis-coplanar transition state geometrical requirements. For example, in the cyclopentyl compound of the above series, the cis-coplanar arrangement of β-hydrogen and the epoxygen is easily attained, whereas trans-coplanarity would involve excessive strain. The cis-coplanarity is attainable only with the acyclic β-proton (Fig. 37) in cyclohexyl compound.

The regioselective nature of exocyclic epoxides opening by strong organic bases has been extensively used in the syntheses of prostaglandins (Fig. 38).

Another aspect of the selectivity of these reactions lies in the non-reactivity of tertiary β-hydrogens. Thus, if the system contains both the primary and the tertiary β-hydrogens, the reaction will proceed by abstracting primary β-hydrogen, even if the geometry of the molecule does not favour this, albeit (slowly and in low yield) (Fig. 39).

Figure 36

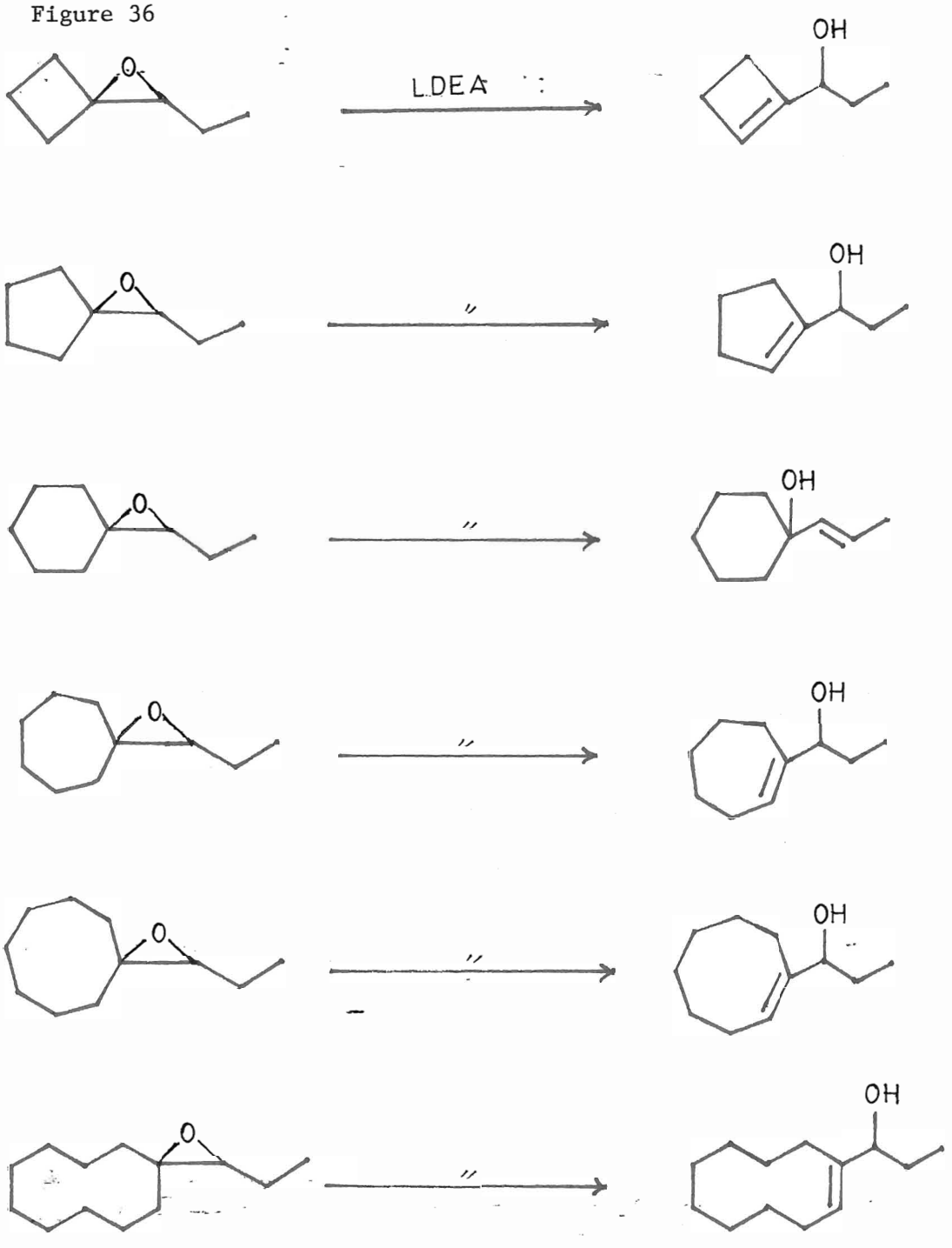


Figure 37

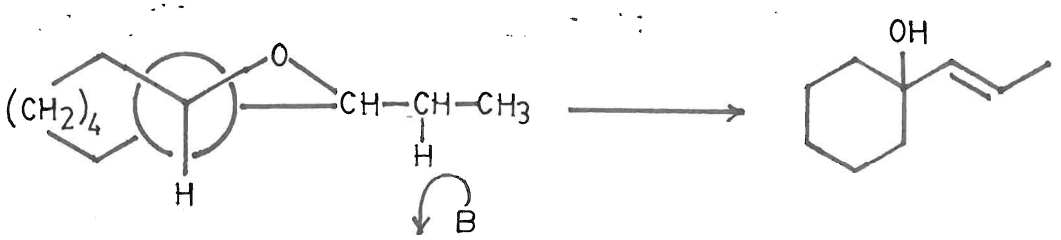


Figure 38

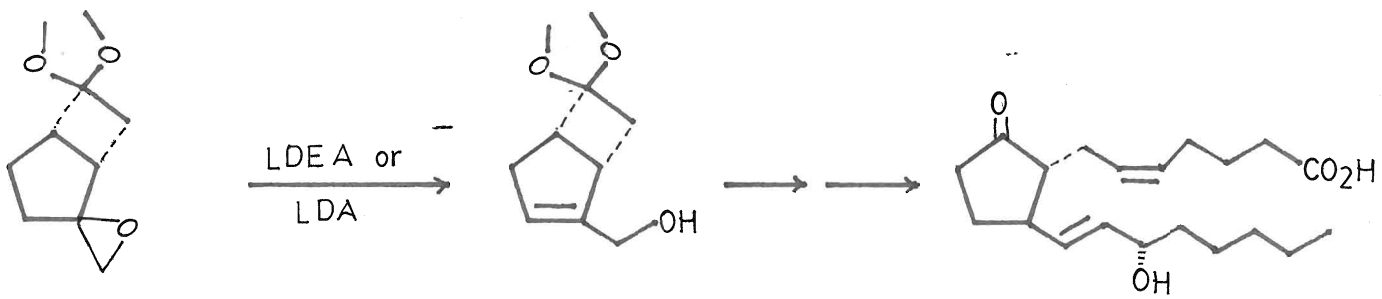
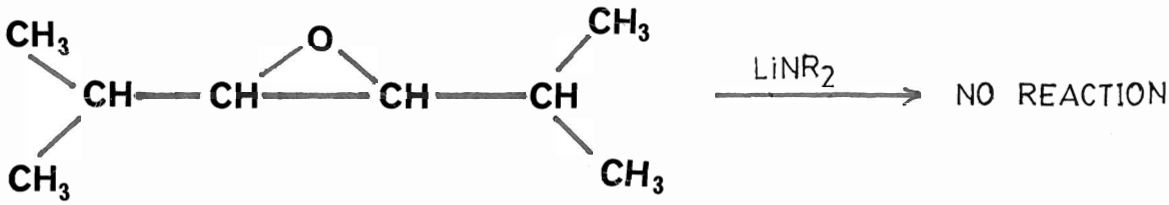
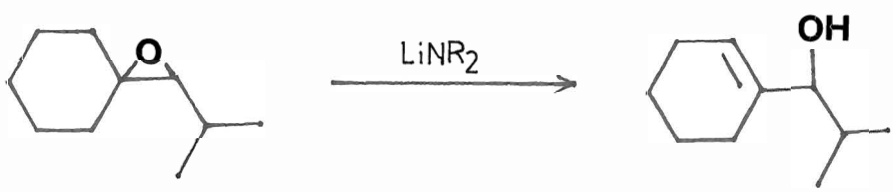


Figure 39



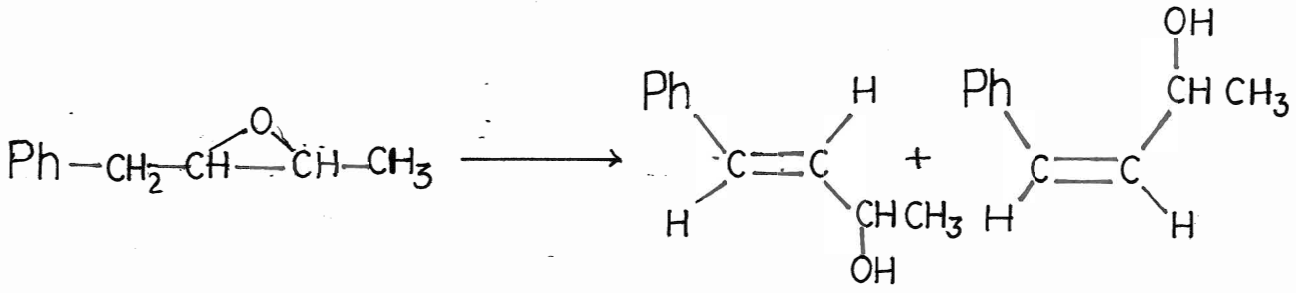


The β-elimination is greatly favoured when epoxides have a β-phenyl substitution,[75] whereas, α-phenyl substitution shows a preference for α-elimination. The β-phenyl substitution has a strong acidifying effect on β-hydrogen. Even competition for abstraction between primary aliphatic and secondary benzylic protons leads to exclusive reaction by the latter pathway. This has been illustrated by the reaction of 1-phenyl-2-butene oxides (113) where no 1-phenyl-3-butene-2-ol is formed. The cis isomer of (113) yielded pure trans-allylic product (114), whereas the trans-isomer of (113) gave 95% of trans-allylic alcohol (114) along with 5% of cis-allylic alcohol (115).

This is interpretable in terms of a syn elimination, considering steric effects in the transition state (Fig. 40).

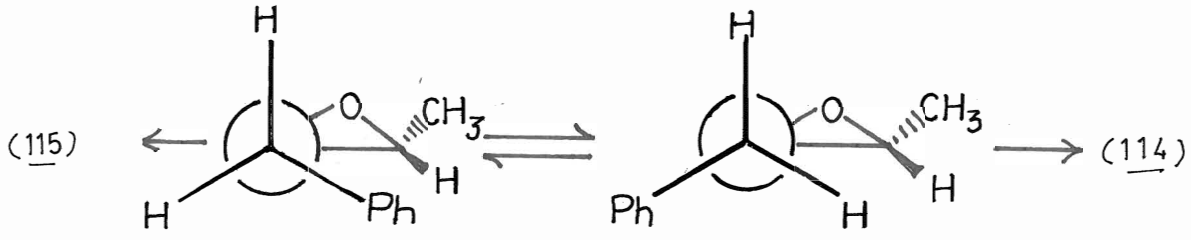
Not only the substituents, but also solvents have been reported to play an important role in deciding the fate of these base-promoted reactions of epoxides.[76]

The suppression of α-elimination during the opening of epoxides by lithium amides can be achieved in many cases by the use of HMPT as the solvent. This solvent effect has been demonstrated by Apparu and Barrelle,[76,77] to promote the formation of allylic alcohols and to suppress the formation of byproducts such as ketones and amino alcohols (Fig. 41). For instance, base-catalyzed rearrangement of cyclo-octene oxide with lithium diethylamide (LDE), in ether, yields bicyclic cyclo-oct-2-ene-ol as the major product, whereas in HMPT, it gives exclusively cyclo-oct-2-ene-ol.

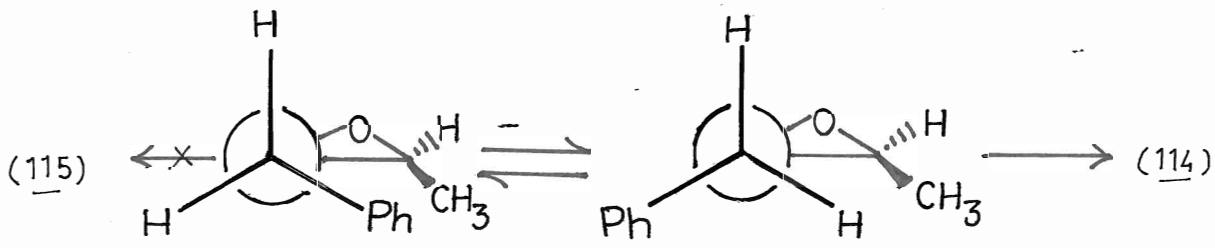


(113)	(114)	(115)
CIS	100%	0%
TRANS	95%	5%

Figure 40

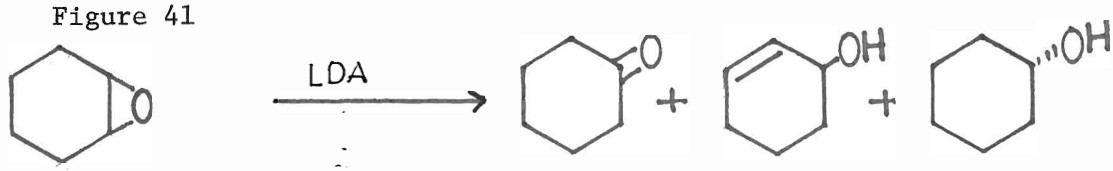


TRANS

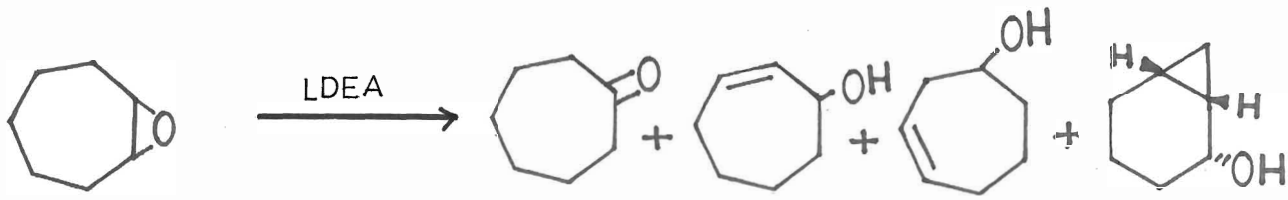


CIS

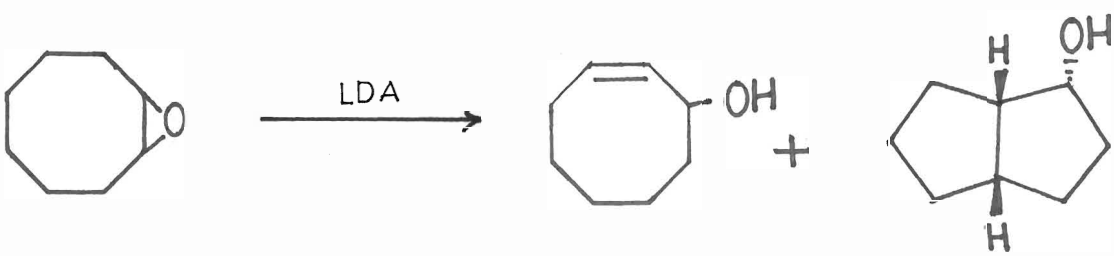
Figure 41



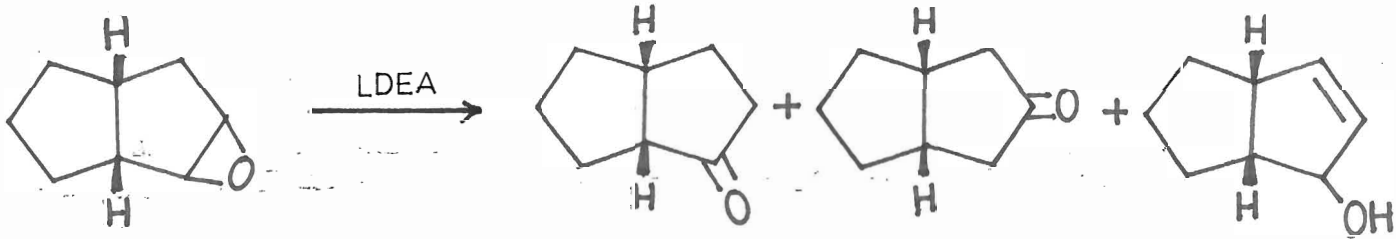
Et_2O	33%	38%	28%
HMPT	–	100%	–



Benzene	36%	23%	–	41%
HMPT	–	82%	18%	–



Et_2O		2%	98%
HMPT	–	100	–



Et_2O	14%	10%	76%
HMPT	–	–	100%

From the study of the reactivity of γ,δ-ethylenic epoxides in the presence of lithium allylamides, in nonpolar solvents (ether, hexane, benzene), their normal behaviour of facile β-elimination has been observed[78] (Fig. 42). In many cases, a total change in the reactivity has been observed when HMPT was used as solvent. The directing effect of HMPT has been found to promote the γ-elimination,[78,79] leading to the formation of 1-hydroxyallyl-2-vinyl cyclopropanes in high yields (Fig. 42). Nevertheless this cyclization needs a favourable epoxide conformation to be attained, otherwise β-elimination is still preferred.

Currently, how HMPT affects the course of reaction is not well understood, however, it has been proposed[77] that HMPT complexes with lithium alkylamides, which in turn brings about the change in the selectivity of base-promoted epoxide rearrangements.

The present research was undertaken to have a better understanding of the base-promoted rearrangements of steroidal epoxides, in both the presence and absence of certain substituents at β and α to the oxirane ring. In addition, another concern was the investigation of simpler stereoselective synthetic routes to allylic alcohols.

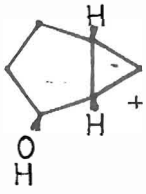
Figure 42

EPOXIDE

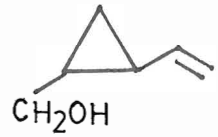
PRODUCTS

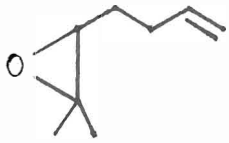
Benzene/Ether

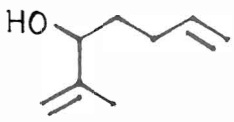
HMPT

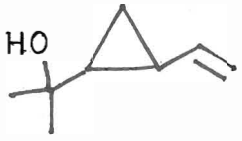


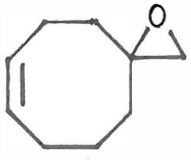
+ amino alcohol

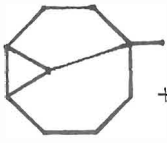




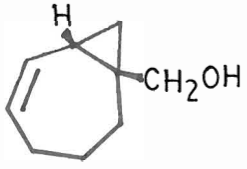


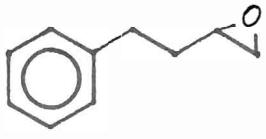




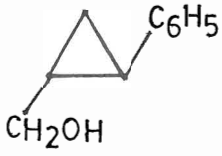


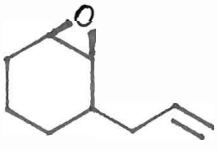
+ amino alcohol

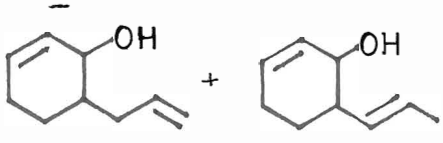




amino alcohol



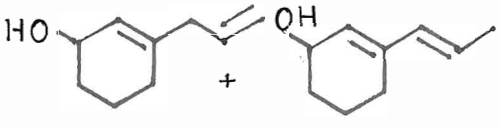


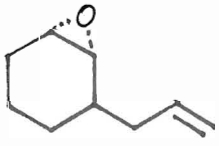


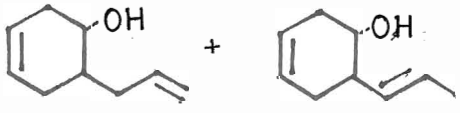
+

+

amino alcohol







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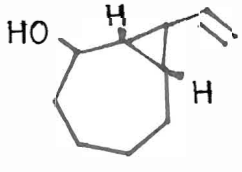


EXPERIMENTAL-I

EXPERIMENTAL-I

Apparatus, Materials and Methods:

Melting points were determined on a Kofler heating stage or on a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra were recorded with a Perkin Elmer 273B grating infrared spectrophotometer or Perkin Elmer 710B infrared spectrophotometer in nujol, KBr disc or chloroform film. The ^{1}H NMR spectra were recorded with a Varian A60, Bruker WP-60 or Bruker WH-400 using $CDCl_3$ (unless otherwise stated) as solvent with TMS as internal standard. Chemical shifts are given in δ scale in ppm. The ^{13}C NMR spectra were recorded with a Bruker WP-60 or a Bruker WH-400. The ^{2}H NMR spectra at 13.8 MHz were recorded with a Bruker WH-90 at 61.402 MHz. ^{2}H NMR spectra with Bruker WH-400 in benzene or chloroform as solvent and benzene-d_6 or $CDCl_3$ as external reference. Mass spectra were obtained with an AEI MS-30 double beam mass spectrometer at about 70 eV. Exact masses and deuterium analyses were determined on an AEI MS-30 double beam mass spectrometer. The isotopic composition of the steroids employed in this study were determined by mass spectrometry. Peak heights were measured and corrected for natural abundance of ^{13}C, and the results calculated from data taken on the average of 5 to 10 scans of the molecular ion regions. Column chromatography was performed on silica gel, neutral alumina or fluorosil. Thin layer chromatography was performed on Merck silica gel 60-F_{254} (0.25 mm thick). The plates were examined

under UV light and sprayed with 80% concentrated sulfuric acid/ethanol (95%) solution and developed at about 110°C. Preparative layer chromatography was performed on Merck silica gel F_{254} (20 x 20, 2.0 mm thick) and plates were examined under UV light or iodine vapour to visualize bands. All the solvents and reagents were purified and dried by standard techniques.[80,81] Elemental analyses were performed by Guelph Chemical Laboratories, Guelph, Ontario, or by Galbraith Laboratories Inc., Knoxville, Tennessee, U. S. A.

Preparation of Steroid Substrates:

5α,6α-Epoxycholestan-3β-ol[82] (2):

To a solution of cholesterol (9.65 g) in methylene chloride (75 mL) was gradually added a solution of m-chloroperbenzoic acid (5.5 g) in methylene chloride (100 mL) over 15 minutes with continuous stirring and the temperature was kept at 25°C. Agitation was continued for 20-30 minutes. Excess peracid was then destroyed by adding 10% sodium sulfite solution until a starch iodide paper test was negative. The organic layer was separated and washed with 5% sodium bicarbonate solution, followed by washing with water and saturated sodium chloride solution, dried over anhydrous sodium sulfate, and evaporated. Crystallization from aqueous acetone (88%) gave 5α,6α-epoxycholestan-3β-ol (9.0 g), m.p. 141-143°C (lit.[82] 142-143°C), ir (nujol) ν_{max}: 3500 cm^{-1}. ^{1}H NMR data are presented in Table 2. ^{13}C NMR data are presented in Table 7. Mass spec. m/z (%): 402 (8.7), 400 (10.6), 385 (11.4), 384 (30.6), 369 (27.9), 366 (14.8), 331 (27.0), 247 (24.3), 245 (15.7), 231 (10.3), 229 (16.7), 213 (11.1), 211 (14.8), 191 (10.0), 175 (15.5).
Acetylation of (2) gave corresponding acetate (2A). ^{1}H NMR data are given in Table 2, and ^{13}C NMR data are given in Table 6.

5β,6β-Epoxycholestan-3β-ol[83] (3):

Cholesteryl acetate (10.07 g) (m.p. 116°C) was dissolved in carbon tetrachloride (20 mL) and cooled to 0°C. To this 250 mL of AcOBr (0.1 M) was added. After five minutes, the resulting solution was shaken with cold

sodium bisulfite solution (5%, 5 mL). The organic layer was washed with water (2 x 50 mL), dried over anhydrous sodium sulfate and concentrated to an oily residue which was crystallized from warm methanol, yielding crude 3β,6β-diacetoxy-5α-bromocholestane (119) (10.5 g) m.p. 78-88°C. Several crystallizations from methanol afforded a pure sample, m.p. 88-90°C (lit.,[83] 89-91°C).

3β,6β-Diacetoxy-5α-bromocholestane (3.0 g) was refluxed for one hour with methanolic sodium hydroxide solution (5%, 80 mL), cooled and neutralized with acetic acid. The reaction mixture was concentrated to about 15 mL under reduced pressure, and water (100 mL) was added, and the precipitate was filtered, washed, dried over anhydrous sodium sulfate, and evaporated. Crsytallization from methanol yielded 5β,6β-epoxycholestan-3β-ol (3) (0.5 g), m.p. 130-132°C (lit.,[85] 131°C).

Ir ν($CHCl_3$ film) ν_{max}: 3500 cm^{-1}. ^1H NMR spectrum is given in Table 2. ^{13}C NMR data are presented in Table 7.

Mass spec: m/z (%): 402 (9.9), 384 (23.0), 269 (20.5), 366 (11.2), 355 (11.0), 331 (6.8), 247 (20.4), 229 (16.5), 149 (24.2), 135 (40.3), 107 (48.6), 95 (90.3).

In another set of experiments, the 3β,6β-diacetoxy-5α-bromocholestane was prepared according to the procedure described by Robinson et al.[84] using lithium acetate and N-bromoacetamide.

On acetylation, the above epoxide gave 5β,6β-epoxycholestan-3β-ol-3-acetate (3A), m.p. 98-100°C (lit.,[82] 102-103°C). ^1H NMR data are given in Table 2, and ^{13}C NMR data are presented in Table 6.

Δ^5-Cholestene[86] (153):

Cholesteryl chloride (10.0 g) was dissolved in isoamyl alcohol (250 mL), then sodium metal (17.3 g) was added in portions to the above boiling solution. All the sodium metal dissolved overnight, and the reaction mixture was then cooled, and remaining bits of sodium metal were destroyed by adding methanol (10 mL) in portions. Then water (150 mL) was added, and the reaction mixture was extracted with ether, the ethereal extract was washed successively with water, 5% hydrochloric acid, water, 10% $NaHCO_3$ solution, water, and finally with saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulfate, and evaporated. Crystallization from methanol yielded Δ^5-cholestene (9.02 g) in about 90% yield, m.p. 94-95°C (lit.,[86] 95°C). ^1H NMR data are given in Table 1. Mass spec, m/z (%): 370 (M^+, 55.2), 355 (40.2), 301 (15.0), 275 (17.1), 257 (25.8), 215 (46.4), 161 (30.8), 247 (28.3), 145 (40.2), 135 (34.1), 109 (52.7).

5α,6α-Epoxycholestane (154)[41]

Δ^5-Cholestene (3.0 g) was dissolved in distilled benzene (100 mL) and m-chloroperbenzoic acid (2.4 g) in benzene (25 mL) was added dropwise with continuous stirring to the Δ^5-cholestene solution over 15 minutes while the temperature was maintained at 20°C. The reaction mixture was stirred for a further 30 minutes, then excess of peracid was destroyed with sodium sulfite solution (10%). Usual workup afforded 5α,6α-epoxycholestane (2.85 g) which on several crystallizations from methanol gave

a pure sample (154), m.p. 73-74°C (lit.,[19] 74-75°C). ^{1}H NMR data are given in Table 2, and ^{13}C NMR data are presented in Table 8.

Mass spec. m/z (%): 386 (M^{+}, 100), 371 (60.3), 368 (80.3), 353 (40.7), 331 (91.4), 273 (30.8), 255 (53.1), 231 (62.2). (Abundances adjusted for m/z 386 as 100%.)

5β,6β-Epoxycholestane (155)[15]

To a solution of Δ^{5}-cholestene (2.22 g) in THF (90 mL) was added a solution of N-bromosuccinimide (2.349 g) in perchloric acid (5%, 20 mL) over 30 minutes with constant stirring. The reaction mixture was left stirring for another 30 minutes, diluted with chloroform (150 mL), neutralized with potassium carbonate solution (5% aqueous, 100 mL) and saturated with sodium chloride to separate the organic layer. The organic layer was washed and dried over anhydrous sodium sulfate.

The residue obtained after the evaporation of the solvent was dissolved in methanol (60 mL) containing sodium hydroxide solution (5%), and heated to 30°C for 30 minutes under refluxing conditions. After the evaporation of the solvent from the reaction mixture in vacuo, the residue obtained was dissolved in chloroform and water. The organic layer separated and was washed with water, dried over anhydrous sodium sulfate and evaporated in vacuo. The crude residue showed the presence of three compounds on tlc. The crude extract, therefore, was chromatographed on silica gel (200 g), eluting with hexane-benzene gradients, gave oily 5β,6β-epoxycholestane (1.40 g), which on repeated crystallization from

aqueous acetone gave the pure sample (155) (1.08 g) m.p. 53-55°C (lit.,[87] 55-57°C). ^{1}H NMR data are given in Table 2, and ^{13}C NMR data are presented in Table 8.

Mass spec. m/z (%): 386 (M^{+}, 35.3), 371 (29.2), 368 (179), 331 (17.1), 273 (19.7), 262 (10.3), 255 (13.6), 247 (21.8), 246 (17.0), 232 (19.9), 231 (66.1), 163 (24.5), 161 (30.8), 259 (20.3), 149 (34.9), 247 (32.9).

On further elution was also separated cholestan-5α-ol-6-one (0.30 g) (157), m.p. 150-152°C crystallized from methanol (lit.,[88] 152-153°C); ir ($CHCl_3$ film) ν_{max}: 3560, 1710 cm^{-1}. ^{1}H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 15.

Mass spec. m/z (%): 402 (M^{+}, 37.7), 382 (49.7), 369 (27.3), 366 (10.5), 366 (10.5), 356 (12.8), 318 (42.2), 271 (10.7), 247 (15.3), 243 (100), 231 (16.4), 230 (13.7), 229 (25.8), 211 (12.4), 199 (22.8), 161 (27.2), 123 (63.6), 112 (52.8).

The third component separated was identified as cholestane-5α,6β-diol (0.09 g) (156), m.p. 121-124°C (lit.,[89] 125.5°C); ir (nujol) ν_{max}: 3600 cm^{-1}; this was identical with an authentic sample (mixed m.p., tlc, ir, ^{1}H NMR, mass spec) prepared according to the procedure described by Tadashi et al.[15] ^{1}H NMR data are given in Table 4 and ^{13}C NMR data are presented in Table 15.

Mass spec. m/z (%): 404 (M^{+}, 0.5), 386 (14.9), 371 (8.9), 386 (27.7), 353 (9.9), 331 (9.4), 255 (13.0), 247 (17.2), 231 (22.8), 213 (14.1), 161 (22.2), 159 (19.9), 149 (19.8), 147 (33.1), 145 (37.6), 109 (50.6), 107 (55.7), 81 (96.2), 55 (100).

Cholest-4-ene-3-one (148)[90]

This compound was prepared according to the procedure described by Eastham and Teranishi[90] from cholesterol, and had m.p. 78-81°C (lit.,[90] m.p. 79.5-80.5°C); ir (nujol) ν_{max}: 1680 cm^{-1}; ^1H NMR data are given in Table 1.

Mass spec. m/z (%): 384 (M^+, 41.7), 369 (10.3), 342 (21.4), 327 (7.2), 299 (10.1), 271 (10.1), 261 (26.6), 229 *48.4), 147 (26.3), 124 (100).

Cholest-5-ene-3,3-ethylene dioxyketal (149)[91]

A stirred mixture of cholest-4-ene-3-one (148) (13.8 g) in distilled benzene *dry, 770 mL) and ethylene glycol (40 mL) was slowly distilled for 15 minutes to remove the traces of water, then p-toluenesulfonic acid monohydrate (0.30 g) was added and the mixture was refluxed for 4.5 hours with continuous removal of water using Dean-Stark separator. Saturated sodium bicarbonate solution (250 mL) was added to the cooled mixture, and the organic layer was separated, washed with water (2 x 100 mL), dried over anhydrous sodium sulfate and evaporated in vacuo. Crystallization from ether-methanol afforded cholest-5-ene-3,3-ethylene dioxyketal (149) (13.4 g) m.p. 132-136°C. Further recrystallization from ether-methanol afforded a pure sample (11.85 g) m.p. 133-134°C (lit.,[91] 133.5-134.5°C). ^1H NMR data are given in Table 1, and ^{13}C NMR data are presented in Table 7.

Mass spec m/z (%): 428 (M^+, 10.3), 384 (11.9), 341 (5.7), 371 (13.9), 269 (11.6), 261 (18.3), 247 (23.4), 229 (73.5).

Epoxidation of cholest-5-ene-3,3-ethylene dioxyketal (149)[92]

A stirred soltuion of cholest-5-ene-3,3-ethylene dioxyketal (149) in methylene chloride (50 mL) was treated with m-chloroperbenzoic acid (7.0 g) in methylene chloride (30 mL) at 0°C, and the solution was kept overnight at 0°C. The usual workup gave a solid (10.0 g), which was chromatographed on fluorosil (300 g). Elution with benzene gave 5β,6β-epoxycholestane-3,3-ethylene dioxyketal (151) which separated from methanol as an amorphous solid (5.31 g), m.p. 128-129°C (lit.,[91] m.p. 126-127°C). ^{1}H NMR data are given in Table 2, and ^{13}C NMR data are presented in Table 7.
Mass spec m/z (%): 444 ($M^{+\cdot}$, 100), 426 (18.2), 416 (28.0), 415 (30.0), 408 (11.0), 382 (17.0), 301 (5.0), 268 (20.0), 251 (11.3). The spectrum was adjusted taking m/z 444 as 100%.

Further elution with ether afforded 5α,6α-epoxycholestan-3,3-ethylene dioxyketal (150) (4.4 g), which separated from methanol as prisms, m.p. 145-146°C (lit.,[91] 118-120°C, lit. [92] 146°C). ^{1}H NMR data are given in Table 2, and ^{13}C NMR data are presented in Table 7.
Mass spec. m/z (%): 444 ($M^{+\cdot}$, 5.1), 222 (1.7), 211 (1.6), 202 (2.8), 200 (2.2), 197 (4.0), 195 (4.2), 99 (75.0).

Δ^{5}-Cholesten-3-one (121)[95]:

The methanol moist dibromocholestan-3-one (124) (25.0 g) prepared according to Fieser[95] from cholesterol dibromide (123) was covered with ether (294.1 mL) and acetic acid (3.6 mL) was added, and the mixture cooled to 0°C. With constant stirring at 10°C, zinc dust (7.0 g) was added in

portions in the course of five minutes with maintenance of a temperature of 15-20°C by occasional cooling. When the exothermic reaction was over, ice bath was removed and stirring was continued for an additional 10 minutes. Then pyridine (10 mL) was added, the resulting complex was filtered by suction and the filter cake was washed well with ether until the filtrate was colorless. The filtrate was washed with pH 7.2 buffer, twice with water, twice with 5% sodium bicarbonate solution, dried over anhydrous sodium sulfate, and evaporated to one-fourth of the volume. Methanol (150 mL) was then added, and the mixture evaporated again to 150 mL. This on crystallization afforded Δ^5-cholesten-3-one (121) (12.3 g), m.p. 124-126°C (lit.,[93] 126-129°C); ir ($CHCl_3$ film) ν_{max}: 1710 cm^{-1}. ^1H NMR data are given in Table 1, ^{13}C NMR data are presented in Table 12. Mass spec. m/z (%): 384 (M^+, 32.0), 342 (9.0), 301 (15.0), 275 (35.0), 271 (30.0), 229 (90.0), 213 (40.0), 187 (42.0), 174 (60.0), 159 (95.0), 147 (72.0), 145 (83.0), 135 (100.0). The fragment ions abundances have been adjusted taking m/z 135 as 100%.

U-Ni-A catalyst:[94]

A 13% aqueous solution of acetic acid (18 mL) was added all at once to precipitated nickel (30.0 g). The reaction vessel was occasionally shaken to prevent the contents from foaming over due to violent evolution of hydrogen. When the reaction subsided, additional aqueous acetic acid solution was added until the solid matter settled, and then the supernatant liquid was decanted. Twice more the acid treatment was carried out using

each time aqueous acetic acid solution (50 mL) and at the end of the acid treatment, a faint green solution could be observed. The nickel catalyst, thus obtained, was washed with water by repeated suspension and decantation until the washings were neutral to litmus. To remove all the traces of water, the catalyst was suspended in cyclohexanol (dry), agitated in an ultrasonic cleaner, and then the supernatant liquid was removed after centrifugation, and the process was repeated several times. The catalyst so obtained contains about 2.4 g of nickel.[95]

Δ^5-Cholestene-3α-ol (epicholesterol) (121)[94]

By using U-Ni-A catalyst (from 30 g of precipitated nickel), cholest-5-ene-3-one (9.0 g) was hydrogenated in cyclohexane (200 mL) at 35°C for three hours with constant stirring (1000 cycles/minute) at 100 kg/cm^2 pressure in a steel autoclave. After the removal of solid matter from the resulting reaction mixture by filtration, evaporation in vacuo gave crude product (8.7 g). The crude product was then chromatographed on silica gel and elution with hexane-ether (9:1) gave epicholesterol (8.2 g), m.p. 139-140°C (lit.,[96] 141°C), ir (KBr) ν_{max}: 3350, 1022, 1007, 993 cm^{-1}. ^{1}H NMR data are given in Table 1, and ^{13}C NMR data are presented in Table 5.

Mass spec. m/z (%): 386 (M^+, 6.6), 368 (22.1), 353 (11.6), 275 (3.9), 260 (5.7), 255 (9.2), 247 (13.5), 229 (10.4).

Also separated was cholesterol (0.21 g), m.p. 146°C identical with authentic sample (^{1}H NMR, Mass).

Acetylation of the epicholesterol afforded Δ^5-cholestene-3α-ol-3-acetate (127), m.p., 84-85°C (lit.,[96] 85°C); ir ($CHCl_3$ film) ν_{max}:

1735 cm^{-1}. ^1H NMR data are given in Table 2, and ^{13}C NMR data are presented in Table 5.

Mass spec. m/z (%): 368 (M^+—CH_3COOH, 100), 353 (24.6), 260 (12.8), 255 (17.6), 247 (23.4), 213 (16.1), 159 (17.0), 147 (43.0), 145 (35.1).

5α,6α-Epoxycholestan-3α-ol (125)[41]

Epicholesterol (2.5 g) was dissolved in methylene chloride (100 mL), to this a solution of m-chloroperbenozic acid (1.45 g) in methylene chloride (50 mL) was added dropwise with constant stirring at room temperature for 15 minutes. Workup as usual afforded the 5α,6α-epoxycholestan-3α-ol (125), (2.31 g), which crystallized from aqueous methanol as needles, m.p. 124-125°C (lit.,[97] m.p. 124°C), ir ($CHCl_3$ film), ν_{max}: 3570 cm^{-1}. ^1H NMR data are given in Table 2, and ^{13}C NMR data are presented in Table 5.

Mass spec. m/z (%): 402 (M^+, 17.0), 400 (10.2), 384 (54.0), 369 (46.0), 366 (27.0), 256 (11.0), 331 (12.1), 271 (10.7), 247 (28.0).

Acetylation of this epoxide gave 3α-acetoxy-5α,6α-epoxycholestane (129), m.p. 106-108°C (lit.,[97] 111-112°C); ir ($CHCl_3$ film) ν_{max}: 1740, 1250, 1235 cm^{-1}. ^1H NMR data are given in Table 2, and ^{13}C NMR data are presented in Table 6.

Mass spec. m/z (%): 444 (M^+, 1.2), 385 (22.1), 384 (71.1), 370 (13.7), 369 (46.7), 368 (17.5), 367 (16.8), 366 (39.2), 356 (20.1), 247 (28.4), 229 (21.0), 211 (25.2). Analysis calculated for $C_{29}H_{48}O_3$: C 78.378, H, 10.810; found: C78.17, H 11.03.

Epoxidation of 3α-acetoxycholest-5-ene (127)

To a solution of 3α-acetoxycholest-5-ene (127) (5.0 g) in methylene chloride (200 mL) was added m-chloroperbenzoic acid (3.0 g) in methylene chloride (50 mL) dropwise in course of 15 minutes at room temperature with constant stirring. The stirring was continued for an additional five hours, at the end of reaction, excess of peracid was destroyed with 5% sodium sulfite solution until the starch iodide paper test was negative. The reaction mixture was successively washed with water (3 x 150 mL), 5% sodium bicarbonate solution (3 x 100 mL), water (2 x 100 mL) and finally with saturated sodium chloride solution, and then dried over anhydrous sodium sulfate, and evaporated *in vacuo*. Crystallization from acetone-hexane afforded 3β,5β-oxidocholestan-6α-ol-6-acetate (131) (1.35 g), m.p. 170-172°C repeated crystallization from ethyl acetate-hexane afforded pure sample of oxetane (131) m.p. 196-197°C, ir ($CHCl_3$ film) ν_{max}: 3500, 1740 cm^{-1}. ^1H NMR data are given in Table 4.
Mass spec. m/z (%): 402 ($M^+ - CH_2CO$, 4.5), 384 (97.3), 376 (22.1), 369 (72.2), 368 (12.0), 367 (18.3), 366 (40.0), 356 (21.0), 355 (15.7), 351 (11.6), 247 (25.9), 229 (29.3), 211 (33.8), 161 (33.8), 135 (68.8), 122 (57.0), 121 (629), 95 (100). Analysis calculated for $C_{29}H_{48}O_3 \cdot H_2O$: C 75.35, H 10.72; found C 75.35, H 10.82.

The mother liquor was concentrated to an oil and dissolved in methanol, stirred with 5% methanolic sodium carbonate solution with slight heating for 10 hours. The solution was then concentrated and thoroughly extracted with methylene chloride, dried, and evaporated. The crude mixture was

chromatographed on silica gel, when elution with hexane-benzene gave 3α-acetoxy-5α,6α-epoxycholestane (129) (0.265 g) m.p. 105-109°C, crystallized from ethyl acetate-hexane. It was found to be identical with the authentic sample. Also separated was 5β,6β-epoxycholestan-3α-ol (126) (0.79 g), m.p. 156-159°C (lit.,[98] 144-146°C); ir (nujol) ν_{max}: 3420, 1350, 1240 cm^{-1}. ^{1}H NMR data are given in Table 2, and ^{13}C NMR data are presented in Table 5.

Mass spec m/z (%): 402 (M^{+}, 12.5), 384 (94.9), 369 (72.3), 367 (19.8), 366 (45.61), 356 (19.8), 355 (15.8), 271 (15.6), 247 (32.5), 229 (33.3), 211 (34.5), 95 (100).

3α-Acetoxy-5β,6β-epoxycholestane (130)

This was prepared by acetylation of 5β,6β-epoxycholestan-3α-ol using pyridine and acetic anhydride. The product, 3α-acetoxy-5β,6β-epoxycholestane (130) was obtained as an oil,[97] efforts to crystallize made it impure. A new tlc spot developed during this, corresponding to 3β,5β-oxidocholestan-6α-ol-6-acetate (131); ir ($CHCl_3$ film) ν_{max}: 1735, 1235, 1020 cm^{-1}.

Mass spec m/z (%): 402 ($M^{+}-CH_2CO$, 12.7), 385 (27.7), 384 (47.8), 370 (14.4), 369 (48.1), 368 (10.3), 366 (22.3), 356 (12.0), 355 (11.0), 271 (13.8), 247 (30.3), 245 (13.9), 229 (30.9).

Rearrangement of 3β,5β-oxidocholestan-6α-ol-6-acetate (131) to 3α-acetoxy-5β,6β-epoxycholestane (130)

The oxetane acetate (131) (0.100 g) in 25 mL round-bottom flask was melted on an oil bath maintained at 200°C at reduced pressure (0.1 mm Hg). The oil so obtained after melting had spectral properties identical with 3α-acetoxy-5β,6β-epoxycholestane (130), but could not be crystallized.

Rearrangement of 3α-acetoxy-5β,6β-epoxycholestane (130) to 3β,5β-oxidocholestan-6α-ol-6-acetate (131)

The epoxide acetate (130) (0.100 g) was dissolved in chloroform (15 mL) and left at room temperature for 20 days. Evaporation of chloroform and crystallization from ethyl acetate-hexane afforded the oxetane acetate (131) (0.06 g). It had spectral properties identical with an authentic sample.

3β,5β-Oxidocholestan-6α-ol (134)

The oxetane acetate (131) (0.075 g) was dissolved in dry ethyl ether (30 mL), and was added to lithium aluminum hydride powder (0.050 g) in ether (25 mL) dropwise under anhydrous conditions, with constant stirring at 0°C. The stirring was continued for an additional four hours. Excess of the reagent was destroyed by adding ethyl acetate (dropwise). The precipitate was filtered and washed with ether. The filtered precipitate was dissolved in sulfuric acid solution (3N, 20 mL), and extracted thrice with ether, the ethereal extracts were combined, washed

with water, dried over anhydrous sodium sulfate. On evaporation in vacuo, it afforded non-crystalline 3β,5β-oxidocholestan-6α-ol (134) (0.05 g), m.p. 160-163°C, ir ($CHCl_3$ film) ν_{max}: 3540 cm^{-1}. ^1H NMR data are given in Table 5.

Mass spec. m/z (%): 402 (M^+, 5.8), 384 (18.1), 369 (13.6), 247 (13.3), 229 (14.9), 211 (10.7), 161 (13.1), 159 (12.0), 149 (18.6), 147 (12.5), 137 (14.5), 95 (50.1), 57 (100). Exact mass, calculated for $C_{27}H_{46}O_2$, 402.350; observed 402.350.

Δ^4-Cholestene (159)[99]

Cholest-4-en-3-one semicarbazone (162)[100] (3.5 g), m.p. 229-232°C (lit.,[99] 234°C) was dissolved in dry toluene (150 mL). To this solution, potassium t-butoxide (2.0 g) was added and the reaction mixture was refluxed under an atmosphere of dry nitrogen, with gas trap, until the evolution of nitrogen ceased (about 60 hours). The reaction mixture was cooled and neutralized with dilute hydrochloride acid, and the organic layer was separated. The aqueous layer was extracted with ether, the ethereal extracts were combined with the toluene layer, washed with water (2 x 150 mL), dried over anhydrous sodium sulfate, and evaporated to a brown gum. Filtration of this gum through an alumina column gave pure cholest-4-ene (159) (1.87 g), m.p. 70-73°C (lit.,[103] 76-78°C). ^1H NMR data are presented in Table 1.

Mass spec. m/z (%): 370 (M^+, 79.4), 356 (14.5), 355 (51.3), 257 (76.7), 247 (10.9), 231 (17.2), 230 (12.8), 216 (16.4), 215 (57.3), 201 (12.2),

161 (25.2), 149 (26.7), 147 (50.5), 135 (52.3), 108 (100).

Epoxidation of Δ^4-cholestene[41] (159)

Δ^4-Cholestene (159) (1.2 g) in methylene chloride (75 mL) was treated with m-chloroperbenzoic acid (0.85 g) in methylene chloride (25 mL) dropwise at 0°C. The reaction mixture was stirred overnight at 0°C. Usual workup afforded the crude product (1.20 g), m.p. 86°C. Fractional crystallization from acetone-water gave 4α,5α-epoxycholestane (160) (0.530 g). Repeated crystallization from acetone (aqueous) gave a pure sample, m.p. 101-102°C (lit.[102] 101-103°C). ^{1}H NMR data are given in Table 3, and ^{13}C NMR data are presented in Table 8.

Mass spec. m/z (%): 386 (M^+, 70.8), 371 (25.6), 370 (18.7), 369 (11.6), 368 (33.5), 358 (11.0), 357 (19.4), 353 (16.7), 255 (16.2), 247 (29.1), 246 (20.3), 232 (35.8), 231 (74.5), 213 (26.0), 147 (58.7), 95 (99.1).

Also separated in fractional crystallization was 4β,5β-epoxycholestene (161) (0.310 g); repeated crystallization gave a pure sample, m.p. 62-64°C (lit. [102] 64-65°C). ^{1}H NMR data are given in Table 3, and ^{13}C NMR data are presented in Table 8.

Mass spec. m/z (%): 386 (M^+, 47.2), 371 (20.6), 370 (19.5), 369 (8.3), 368 (21.2), 357 (13.1), 355 (13.5), 353 (11.8), 247 (21.9), 232 (23.2), 231 (51.9), 215 (21.7), 163 (20.3), 161 (23.6), 247 (53.0).

Δ^4-Cholesten-3β-ol (163)[103]

A suspension of sodium borohydride (1.0 g) in methanol (10 mL) was added to a solution of cholestenone (148) (3.86 g) in methanol (200 mL) and the reaction mixture was stirred for 48 hours at room temperature. Excess of the reagent was destroyed with a few drops of dilute acetic acid. The reaction mixture was concentrated to 25 mL, and crystallization afforded Δ^4-cholesten-3β-ol (163) (3.8 g), m.p. 126-128°C. Recrystallization from ethyl acetate-methanol gave a pure sample (163), m.p. 130-132°C (lit.,[104] m.p. 131-132°C). ^{1}H NMR data are given in Table 1, and ^{13}C NMR data are presented in Table 12.

Mass spec. m/z (%): 386 (M^+, 2.2), 384 (3.8), 368 (21.9), 247 (10.6), 211 (11.9), 179 (13.4), 161 (10.3), 158 (11.4), 147 (21.9), 107 (21.7), 105 (55.6), 55 (100).

Acetylation of the above product gave cholest-4-en-3β-ol-3-acetate (165), m.p. 84-86°C (lit.,[105] 87-88°C). ^{1}H NMR data are given in Table, and ^{13}C NMR data are presented in Table 12.

Mass spec. m/z (%): 428 (M^+, 1.2), 402 (4.5), 368 (100), 353 (25.0), 255 (33.4), 247 (23.2), 213 (21.3), 149 (23.4), 147 (53.0), 145 (34.5), 135 (28.1), 121 (23.9), 106 (47.0), 105 (57.2).

4β,5β-Epoxycholestan-3β-ol (164)[106]

Δ^4-Cholesten-3β-ol (2.4 g) in methylene chloride (75 mL) was treated with m-chloroperbenzoic acid (1.3 g) in methylene chloride (25 mL), dropwise, at 0°C, with constant stirring, in the course of 15 minutes.

The stirring was then continued overnight. Workup as usual gave non-crystalline 4β,5β-epoxycholestan-3β-ol (164) (2.0 g), m.p. 94-96°C (lit.,[87] 95-96°C). ^{1}H NMR data are given in Table 3, and ^{13}C NMR data are presented in Table 11.

Mass spec. m/z (%): 402 (M^{+}, 7.8), 400 (9.3), 384 (35.7), 332 (87.2), 247 (77.6), 231 (32.2), 229 (52.1), 217 (36.8), 215 (30.7), 301 (39.7), 175 (45.7), 173 (32.9), 163 (32.6), 161 (50.2), 160 (32.9), 149 (63.4), 147 (83.8), 135 (80.6), 107 (84.9).

4α,5α-Epoxycholesten-3β-ol (167)[106]

3β-Acetoxycholest-4-ene (165) (0.7 g) in methylene chloride (50 mL) was treated dropwise with a solution of m-chloroperbenzoic acid (0.30 g) in methylene chloride (25 mL) at 0°C with constant stirring. The stirring was continued overnight at room temperature. Workup as usual afforded 4α,5α-epoxycholesten-3β-ol-3-acetate (166) (0.65 g), m.p. 112-115°C from methanol (lit.,[106] 116-117°C). ^{1}H NMR data are given in Table 3, and ^{13}C NMR data are presented in Table 11.

Mass spec. m/z (%): 402 (M^{+}, 9.0), 384 (8.9), 368 (16.9), 333 (25.0), 332 (100), 317 (19.6), 247 (18.3), 229 (10.6), 219 (10.8), 201 (19.6).

The epoxyacetate (166) (0.5 g) was dissolved in benzene (2 mL), diluted with methanol (150 mL) and a small amount of water (2 mL) was added. To this solution potassium carbonate (10.0 g) was added and the slurry mixture was stirred for six hours at room temperature. Potassium carbonate was filtered off, the filtrate was concentrated and thoroughly

extracted with chloroform. The organic extract was dried over anhydrous sodium sulfate, evaporated in vacuo, and crystallization from aqueous methanol gave 4α,5α-epoxycholestan-3β-ol (167) (0.37 g), m.p. 132-135°C (lit.,[107] 136-137°C). ^{1}H NMR data are given in Table 3, and ^{13}C NMR data are presented in Table 11.

Mass spec. m/z (%): 402 (M^{+}, 9.0), 400 (4.2), 385 (8.9), 369 (16.9), 353 (7.4), 332 (100), 317 (19.6), 314 (9.6), 247 (18.3), 229 (10.6), 219 (10.8), 201 (19.6), 177 (10.6), 175 (17.9), 161 (15.3), 159 (15.5), 147 (32.3).

4β,5β-Epoxycholestan-3-one (168)[108]

4β,5β-Epoxycholestan-3-one (168) was prepared by the action of alkaline hydrogen peroxide on cholestenone. This epoxyketone was crystallized from chloroform-methanol, m.p. 118-119°C (lit.,[108] 116-117°C), ir (nujol) ν_{max}: 1710, 1247 cm^{-1}. ^{1}H NMR data are given in Table 3.

Mass spec. m/z (%): 400 (M^{+}, 55.0), 385 (28.5), 384 (35.0), 382 (45.2), 372 (46.1), 357 (32.1), 342 (19.6), 328 (61.3), 287 (41.8), 269 (20.1), 261 (38.5), 247 (43.9).

4β,5β-Epoxycholestan-3α-ol (169)[107]

A solution of 4β,5β-epoxycholestan-3-one (0.40 g) in 80% dioxane-water (28 mL) was treated with sodium borohydride (0.10 g) in the same solvent (8 mL). The mixture was kept for 48 hours at room temperature, and the excess of reagent was destroyed by adding dilute acetic acid

(5 drops). The reaction mixture was poured into ice cold water, and the precipitate collected, m.p. 135-148°C. This was chromatographed on neutral alumina, and elution with benzene gave 4β,5β-epoxycholestan-3α-ol (169) (0.28 g) which on crystallization from ethyl acetate-hexane had m.p. 158-159°C (lit. [106] 158-159°C), ir ($CHCl_3$ film) ν_{max}: 3500 cm^{-1}. ^1H NMR data are given in Table 3, and ^{13}C NMR data are presented in Table 11.

Mass spec. m/z (%): 402 (M^+, 8.2), 400 (4.3), 384 (10.9), 369 (9.1), 368 (6.9), 332 (100), 317 (20.0), 347 (26.4), 229 (17.8), 201 (24.3), 174 (30.5), 147 (38.0).

5α-Cholestan-3β,5,6β-triol-5-acetate (132)

5β,6β-Epoxycholestan-3β-ol (3) (0.5 g) was dissolved in acetic acid (glacial, 50 mL) in a conical flask, sodium acetate (10.0 g) was added to it, and the reaction mixture was warmed for about 20 minutes. The reaction mixture was warmed for about 20 minutes and it was then cooled to room temperature and left as such for one hour. Acetic acid was evaporated *in vacuo*, and the residue was diluted with water (100 mL). The product was thoroughly extracted with chloroform (3 x 150 mL), washed with water (3 x 100 mL), finally with saturated sodium chloride solution, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The crude product was chromatographed on neutral alumina and eluted with benzene-ether (9:1) to give 5α-cholestan-3β,5,6β-triol-5-acetate (132) (0.35 g), crystallized from acetone-hexane, m.p. 171-175°C (lit.,[109] 170°C); ir (nujol) ν_{max}:

3600, 1740 cm^{-1}. ^{1}H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 16.

Mass spec. m/z (%): 402 (M^{+}-CH_3COOH, 29.2), 387 (20.3), 384 (53.1), 369 (47.6), 367 (10.7), 366 (22.2), 356 (13.9), 355 (13.0), 351 (10.5), 331 (14.1), 271 (14.0), 247 (35.0), 245 (14.7), 229 (23.9), 211 (20.1), 125 (52.0), 121 (50.6), 107 (63.4), 95 (100).

5α-Cholestan-3-one (180)

To a solution of 5α-cholestan-3β-ol (179) (5.0 g) in acetone (100 mL) and ethylmethyl ketone (100 mL), was added Jones reagent (6.5 mL) dropwise with constant stirring over the period of 15 minutes at room temperature. The reaction mixture was stirred for an additional 15 minutes, and the excess of the reagent was destroyed by adding a few drops of isopropanol. The resulting reaction mixture was then diluted with water (150 mL) and was thoroughly extracted with ether. The organic layer was washed with water, dried over anhydrous sodium sulfate, and evaporated in vacuo. On crystallization from methanol, 5α-cholestan-3-one (180) (4.63 g) was obtained, m.p. 127-129°C (lit.,[110] 129°), ir ($CHCl_3$ film) ν_{max}: 1718 cm^{-1}. ^{1}H NMR data are given in Table 1.

Mass spec. m/z (%): 386 (M^{+}, 24.6), 371 (11.8), 357 (1.9), 231 (100), 217 (30.9), 163 (23.3), 149 (11.1), 147 (10.6), 135 (12.5), 123 (23.7), 109 (27.8), 95 (37.1).

5α-Cholestan-3,3-ethylene dioxyketal (181)

Cholestan-3-one (4.0 g) was dissolved in ethylene glycol (250 mL) in a round bottom flask equipped with magnetic stirrer and a thermometer. To this p-toluenesulfonic acid monohydrate (0.1 g) was introduced and the reaction mixture was slowly distilled over four hours at 70-78°C (2-1 mm Hg). After two hours, the mixture turned slightly pink. When most of the ethylene glycol was distilled off, sodium hydroxide solution (10%, alcoholic) was added to this turbid mixture, which was then poured onto water (about 300 mL). The precipitate was collected, washed with water, crystallization from petroleum ether (60-110°C)-methanol and gave 5α-cholestane-3,3-ethylene dioxyketal (101) (4.0 g), m.p. 112-114°C (lit.,[111] 113°C). ^{1}H NMR data are given in Table 1.
Mass spec. m/z (%): 430 (M^+, 5.6), 386 (7.1), 371 (3.3), 353 (0.7), 301 (0.9), 263 (1.7), 246 (3.1), 232 (15.0), 231 (28.1), 125 (39.0), 99 (100).

Attempted mesylation of Δ^4-cholesten-3β-ol (163)

A solution of Δ^4-cholesten-3β-ol (0.5 g) in methylene chloride (75 mL, dry), containing triethylamine (0.5 mL, dry) was cooled to -20°C. Mesyl chloride (0.5 mL) was added to the above stirred solution, and the stirring was continued for two hours at -15°C. The resulting reaction mixture was evaporated at room temperature in vacuo, the oily material left after evaporation was dissolved in methylene chloride (100 mL) and washed twice with 1 N HCl solution, with pH 6.86 buffer (2 x 50 mL), finally with saturated sodium chloride solution, and dried over anhydrous

sodium sulfate. The solvent was evaporated at room temperature in vacuo. Crystallization from ether-ethanol yielded $\Delta^{3,5}$-cholestadiene (172) (0.27 g) m.p. 78°C (lit.,[112] 78-79°C). ^{1}H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 13.

Mass spec. m/z (%): 368 (M^+, 100), 353 (33.5), 326 (4.0), 283 (3.2), 260 (26.0), 255 (26.61), 247 (33.1), 213 (26.0).

Reduction of Δ^4-cholesten-3-one with diisobutylaluminum hydride (DIBAL):

To a solution of cholestenone (148) (0.1 g) in dry toluene (25 mL), a solution of DIBAL (25%, 0.5 mL) was added dropwise with stirring over 10 minutes. The stirring was continued for an additional hour at 0°C, then the excess of the reagent was destroyed by adding water (10 mL). The reaction mixture was neutralized with dilute HCl solution, and extracted with ethyl acetate. The organic extract was washed with water (3 x 50 mL), saturated sodium chloride solution (50 mL) and dried over anhydrous sodium sulfate. The extract was evaporated in vacuo and crystallization from methanol afforded Δ^4-cholesten-3β-ol (0.61 g), m.p. 130-135°C. It was identified by tlc and spectral comparison with the authentic sample. The ^{1}H NMR of the crude product indicated the formation of Δ^4-cholesten-3α-ol (170) in less than 10% yield. This therefore was not isolated for further studies.

Attempted preparation of cholesteryl chloride (152)[114]

To a solution of cholesterol (10.0 g) in pyridine (20 mL), thionyl chloride (10 mL) was rapidly added with ice cooling. The reaction mixture

was cooled, poured into ether and diluted with equal volume of water. Saturated sodium bicarbonate solution was slowly added to the above reaction mixture, until the latter was neutral to litmus. The organic phase was separated and the aqueous layer was thoroughly extracted with ether. The combined ethereal extract was washed with water, dried over anhydrous sodium sulfate and evaporated in vacuo. Crystallization from acetone-hexane afforded α-cholesteryl chloride (158) (5.3 g) m.p. 167-179°C (lit.,[113] 95°C; lit.[114] 116°C). ^{1}H NMR data are presented in Table 1, and ^{13}C NMR data are presented in Table 13.

Mass spec. m/z (%): 368 (M^{+}-HCl 77.3), 353 (44.8), 201 (17.3), 275 (26.0), 261 (10.6), 260 (25.0), 255 (47.2), 247 (51.7), 231 (20.3), 213 (54.7), 200 (22.7), 199 (23.2), 190 (43.3), 196 (43.8), 173 (26.7), 263 (34.8), 162 (48.2), 159 (54.9).

Reactions of steroidal epoxides with strong organic bases:

With potassium t-butoxide:

General procedure:

The solution of steroidal epoxide (0.1 g) in diethyl ether or tetrahydrofuran (dry, 10 mL) was added to the freshly prepared potassium t-butoxide in t-butanol (0.25 g, potassium metal in 100 mL of t-butanol), and the reaction mixture was refluxed under dry nitrogen for three days, and monitored by tlc from time to time. Then the resulting reaction mixture was cooled to room temperature, diluted with water (100 mL), neutralized with hydrochloric acid solution (1 N), thoroughly extracted

with ether, the extract dried over anhydrous sodium sulfate and evaporated in vacuo.

None of the epoxides employed in the present study showed any detectable product formation, and only the corresponding starting materials were isolated and were identified by comparing tlc and spectral data (m.p., ^{1}H NMR, mass spec.) with authentic samples.

With lithium di-isopropyl amide (LDA):

General procedure:

A four-necked round bottom flask (250 mL) fitted with condenser and magnetic stirrer was flushed with dry nitrogen and was kept under its continuous, steady flow for at least three hours prior to the start of the reaction. The tetrahydrofuran (THF) (100 mL, dry) was introduced to the above flask through a rubber septum, with a hypodermic syringe and the flask was cooled to -20°C. A solution of methyl lithium (3.30 mL, 1.56 M in ether) was introduced into the flask using a syringe, and then di-isopropylamine (0.8 mL, dry) was added dropwise with constant stirring during the course of 15 minutes. The stirring was continued for an additional 15 minutes, and the temperature was not allowed to rise above -15°C. The reaction vessel was then cooled to -78°C (using dry ice) and the solution of the required epoxide (0.10 g) in tetrahydroduran (THF) (5 mL) was introduced with syringe through the rubber septum. The resulting reaction mixture was stirred at -78°C for 12 hours; then the temperature was slowly raised to -10°C and the stirring was continued for three days. The reaction was monitored with tlc from time to time. At

the end of reaction, the excess of the reagent was destroyed by adding butanol (2 mL) and the mixture then neutralized with hydrochloric acid solution (1N). The resulting reaction mixture was diluted with water (100 mL) and was thoroughly extracted with chloroform. The organic layer was washed with saturated ammonium chloride solution (3 x 50 mL), water (2 x 100 mL), saturated sodium chloride solution (50 mL), dried over anhydrous sodium sulfate and evaporated *in vacuo*.

None of the epoxides employed in the present investigation reacted, only the corresponding starting materials were re-isolated, and were compared with their corresponding authentic samples (m.p., ^{1}H NMR, mass spec.).

Reaction of o-toluic acid with LDA:[115]

The solution of methyl lithium (5.33 mL, 1.56 M in ether) was added to a round bottom flask fitted with condenser and magnetic stirrer, maintained under a continuous, steady stream of dry nitrogen. The flask was cooled to -5°C, and di-isopropyl amine (1.5 mL, dry) was introduced dropwise with stirring into the above flask with a hypodermic syringe. The stirring was continued for an additional 15 minutes, then THF (100 mL) was added and a solution of o-toluic acid (0.5 g) in THF (3 mL, dry) was added slowly using the hypodermic syringe. The stirring was continued, the colour of the solution changed to deep red in one hour, and the reaction mixture was stirred for a further 30 minutes. To this, 1-bromobutane (2 mL) was added dropwise with syringe and the reaction mixture was stirred for an additional 30 minutes. At the end of the

reaction, the excess of the reagent was destroyed with butanol (2 mL), the mixture neutralized with hydrochloric acid solution (1 N), washed with saturated ammonium chloride solution, and water. The reaction mixture was thoroughly extracted with chloroform, the extract was dried over anhydrous sodium sulfate and evaporated *in vacuo*. The oil, so obtained, on GC-mass spec showed at least 95% formation of o-n-pentylbenzoic acid.

With n-butyllithium:

General procedure:

In a four-necked round bottom flask (250 mL) equipped with condenser, magnetic stirrer, rubber septum, and maintained under continuous slow stream of dry nitrogen, was added a solution of steroidal epoxide (0.2 g) in THF (20 mL, dry). The reaction vessel was cooled to 0°C and a solution of n-butyllithium (3 mL, 2.4 M in hexane) was added dropwise from a hypodermic syringe with stirring. The stirring was continued at 0°C and the reaction was monitored with tlc. The temperature was raised to room temperature, if no product formed at 0°C, and stirring was continued for three days. At the end of the reaction time, the excess of the reagent was destroyed with 2-propanol (2 mL). The resulting reaction was diluted with water (100 mL), neutralized with hydrochloric acid solution (5%) and thoroughly extracted with chloroform. The chloroform extract was washed successively with water (2 x 100 mL), sodium bicarbonate solution (10%, 100 mL), water (2 x 100 mL), finally with saturated sodium chloride solution (50 mL), dried over anhydrous sodium sulfate and evaporated *in*

vacuo. The resulting crude product was chromatographed on silica gel or on neutral alumina.

Among 5,6-epoxy steroids employed in the present study, only 5α,6α-epoxycholestane-3,3-ethylene dioxyketal (150) and 5β,6β-epoxycholestan-3,3-ethylene dioxyketal (151) gave some products with n-butyl lithium. The re-isolated starting epoxides were compared with their authentic samples (m.p., tlc, ^{1}H NMR, mass spec).

Reaction of 5α,6α-epoxycholestan-3,3-ethylene dioxyketal (150) with n-butyllithium (n-BuLi):

To a solution of 5α,6α-epoxycholestan-3,3-ethylene dioxyketal (150) (0.5 g) in tetrahydrofuran (25 mL, dry), under the dry setup described above, was added a solution of n-butyllithium (6.0 mL, 2.4 M in hexane) dropwise with stirring at 0°C. After 30 minutes, the temperature of the reaction vessel was raised to the room temperature and stirring was continued for additional two hours. At the end of the reaction, the excess of the reagent was destroyed with 2-propanol (2 mL), and the mixture diluted with pH 6.86 buffer (200 mL). The reaction mixture was extracted several times with chloroform, washed successively with pH 6.86 buffer (2 x 100 mL), water (2 x 100 mL), finally with saturated sodium chloride solution (50 mL), dried over anhydrous sodium sulfate, and evaporated to an oil. The ^{1}H NMR of the oil showed the presence of 3,3-ethylenedioxy-ketal cholest-4-en-6α-ol (173) and included the signals at δ 6.3 (s, 1H, C-4 olefinic H), 4.02 (br s, 4H, $-O-CH_2-CH_2-O-$), 4.15 (m, 1H, C6 H, visible as hump

from the signal at 4.02), 3.05 (br s, 1H, C6 OH, disappears on shaking with D_2O).

Mass Spec. m/z (%): 444 (M^+, 67.7), 427 (24.0), 426 (38.0), 401 (39.1), 400 (100), 398 (52.2), 385 (29.4), 384 (20.9), 383 (31.1), 371 (28.4), 370 (39.7), 331 (50.9), 287 (31.0), 285 (30.0), 247 (72.4), 245 (50.7), 243 (65.2).

The above oil was dissolved in chloroform (100 mL), to this hydrochloric acid solution (1 M, 10 mL) was added and the reaction mixture was stirred for two hours at room temperature. The organic layer was separated, washed successively with pH 6.86 buffer (2 x 100 mL), water (2 x 100 mL), finally with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and evaporated in vacuo. Crystallization from benzene-hexane afforded Δ^4-cholesten-3-on-6α-ol (174) (0.210 g), m.p. 152-154°C (lit.,[116] 150°C), ir ($CHCl_3$ film) ν_{max}: 3400, 1670 cm^{-1}. ^1H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 10.

Mass spec. m/z (%): 400 (M^+, 80.4), 385 (17.1), 382 (13.77), 331 (100), 287 (18.0), 245 (32.0), 231 (11.5).

Reaction of 5β,6β-epoxycholestan-3,3-ethylene dioxyketal (151) with n-butyllithium:

To a solution of 5β,6β-epoxycholestan-3,3-ethylene dioxyketal (151) (0.5 g) in tetrahydrofuran (30 mL, dry) in a four-necked flask, under the dry setup described in general proceudre, was added a solution of

n-butyllithium (5 mL, 2.4 M in hexane) using a hypodermic syringe, with stirring, at 0°C. The reaction mixture was stirred for two hours at room temperature, and workup as usual then afforded a mixture of two compounds, which were chromatographed on silica gel. Elution with benzene-ether (90:10) gave 3β-n-butyl-5β,6β-epoxycholestan-3α-ol (176) (0.05 g), m.p. 140-141°C (crystallized from methanol), ir ($CHCl_3$ film) ν_{max}: 3500 cm^{-1}. ^1H NMR data are given in Table 4.

Mass spec m/z (%): 458 (M^+, 4.2), 443 (2.5), 442 (3.2), 441 (16.9), 440 (46.7), 425 (25.7), 423 (13.3), 422 (19.4), 401 (21.2), 383 (11.2), 247 (12.3), 189 (11.2), 177 (16.3), 175 (20.7), 163 (19.6), 161 (18.3), 149 (31.6), 147 (20.5), 135 (29.7), 121 (30.5), 95 (60.2).

Analysis, calculated for $C_{31}H_{54}O_2$: C 81.22, H 11.79; found C 80.87, H 11.52. (Exact mass observed, 458.395; calculated for $C_{31}H_{54}O_2$, 458.412).

On further elution with increasing polarity (ether-methanol) also separated 3β-n-butylcholest-4-ene-3α,6β-diol (175) (0.38 g), crystallized from aqueous acetone (80%), m.p. 102-105°C.

^1H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 9.

Mass spec.m/z (%): 440 (M^+-H_2O, 36.3), 438 (9.1), 425 (17.5), 424 (21.5), 423 (34.3), 422 (63.4), 412 (11.3), 401 (33.0), 383 (10.6), 247 (20.7), 215 (10.1), 213 (13.1), 201 (12.0), 199 (18.5), 175 (69.6), 119 (40.9), 107 (43.9), 57 (100).

Analysis calculated for $C_{31}H_{54}O_2$: C 81.22, H 11.79; found C 81.18, H 11.71 (Exact mass observed for $C_{31}H_{54}O_2-H_2O$, 440.402; observed 440.410).

3β-n-Butylcholest-4-en-3α,6β-diol-6-acetate (175A)

3β-n-Butylcholest-4-en-3α,6β-diol (0.1 g) was dissolved in pyridine (1.5 mL) and into it acetic anhydride (0.5 mL) was added. The reaction mixture was left at room temperature for 20 hours, then poured onto crushed ice while stirring. The precipitate collected by filtration was thoroughly washed with water. Crystallization from aqueous methanol afforded 3β-n-butylcholest-4-en-3α,6β-diol-6-acetate (175A) (0.07 g), m.p. 87-88°C, ir ($CHCl_3$ film) ν_{max}: 3600, 1740 cm^{-1}. ^1H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 9.
Mass spec. m/z (%): 443 ($M^{\cdot+}-C_4H_9$, 24.4), 422 (21.9), 401 (14.2), 384 (20.1), 383 (20.5), 174 (24.5), 149 (11.8), 135 (13.7), 119 (15.7), 109 (19.7), 97, (35.7), 95 (33.5), 81 (33.9), 71 (45.7), 69 (57.7), 57 (93.5), 56 (40.9), 55 (100).
Analysis, calculated for $C_{33}H_{56}O_3$: C79.20, H 11.20; found, C79.19, H 11.20.

Reaction of 5α-cholestan-3,3-ethylene dioxyketal (181) with n-butyllithium (n-BuLi):

To a solution of 5α-cholestan-3,3-ethylene dioxyketal (181) (0.5 g) in tetrahydrofuran (20 mL, dry) was added a solution of n-butyllithium (6.0 mL, 2.4 M in hexane) according to the procedure described previously, and the reaction mixture was stirred for two hours at room temperature. Workup as usual gave a mixture of two compounds which were chromatographed on neutral alumina. Elution with hexane-benzene (95:5) gave

3β-n-butyl-5α-cholestan-3α-ol (182) (0.320 g), crystallized from aqueous methanol, m.p. 124-125°C. ^{1}H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 9.

Mass spec. m/z (%): 427 (M^{+}-OH, 24.0), 412 (18.2), 388 (23.2), 376 (26.2), 315 (10.2), 271 (23.6), 229 (10.4), 161 (38.8), 147 (31.2), 137 (15.3), 135 (22.0), 121 (30.2), 81 (100).

Analysis calculated for $C_{31}H_{56}O$: C 83.78, H 12.61; found: C 83.789, H 12.92.

Further elution with hexane-benzene (90:10) gave 3α-n-butyl-5α-cholestan-3β-ol (183) (0.09 g), crystallized from aqueous methanol, m.p. 53°C, ir ($CHCl_3$ film) ν_{max}: 3400, 1480, 1390, 1000 cm^{-1}. ^{1}H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 9.

Mass spec. m/z (%): 444 (M^{+}, 0.4), 426 (36.6), 411 (22.8), 387 (40.0), 369 (29.1), 316 (26.0), 315 (11.9), 286 (11.1), 273 (10.5), 272 (16.7), 271 (35.7), 229 (10.4), 203 (31.8), 163 (34.9), 161 (37.1), 147 (33.9), 107 (56.2), 95 (75.5), 81 (100).

Exact mass, calculated for $C_{31}H_{56}O$, 444.433; observed 444.437.

Reaction of 5α-cholestan-3,3-ethylene dioxyketal (181) with methyllithium (MeLi):

To a solution of 5α-cholestan-3,3-ethylene dioxyketal (181) (0.5 g) in tetrahydrofuran (25 mL, dry) was added a solution of methyllithium (6.0 mL, 1.56 M in ether) according to the procedure described previously, and the reaction mixture was stirred at room temperature for four days. Workup as usual afforded a mixture containing two compounds of low R_f

values, along with unreacted starting material. The crude mixture was chromatographed on neutral alumina, when elution with hexane gave starting material (0.2 g) identical with the authentic sample (m.p., ^{1}H NMR, mass spec.). Further elution gave 3β-methyl-5α-cholestan-3α-ol (184) (0.15 g), crystallized from methanol, m.p. 125-127°C (lit.,[111] 126-127°C). ^{1}H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 10.
Mass spec. m/z (%): 402 (M^+, 12.0), 385 (12.3), 384 (38.9), 370 (13.9), 369 (43.9), 376 (32.1), 271 (12.3), 247 (12.2), 244 (19.4), 231 (30.3), 229 (96.7), 203 (22.3), 161 (57.3), 149 (56.6), 122 (56.6), 121 (57.6), 57 (100).

Further elution with hexane-benzene (95:5) gave 3α-methyl-5α-cholestan-3β-ol (185) (0.13 g), crystallized from aqueous acetic acid, m.p. 145-147°C (lit.,[117] 147-149°C). ^{1}H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 10.
Mass spec. m/z (%): 402 (M^+, 9.0), 385 (10.4), 384 (33.2), 370 (10.0), 369 (29.7), 316 (20.5), 244 (14.0), 231 (21.3), 230 (36.7), 229 (73.4), 215 (13.9), 203 (15.2), 149 (25.3), 121 (48.9). The isolated ratio of these two alcohols (α and β) is 1.15:1.

With lithiumdiethyl amide (LDEA):

Reaction of 5α,6α-epoxycholestan-3β-ol (2) with LDEA

To a solution of diethyl amine (2.0 mL, dry) in ether (100 mL, dry), under the setup described previously, was added a solution of n-butyl-lithium (5.16 mL, 2.4 M in hexane) dropwise with constant stirring in

course of 15 minutes at room temperature. The stirring was continued for an additional 15 minutes, then a solution of 5α,6α-epoxycholestan-3β-ol (2) (0.30 g) in tetrahydrofuran (5 mL, dry) was introduced into the above flask using hypodermic syringe, and the reaction was then refluxed gently for 24 hours. At the end of the reaction, the excess of the reagent was destroyed by dropwise addition of water (10 mL), and the mixture then washed with saturated ammonium chloride solution (2 x 75 mL). The reaction mixture was then thoroughly extracted with chloroform, washed with water (2 x 100 mL), pH 6.86 buffer (100 mL), and finally with saturated sodium chloride solution. The organic layer after drying on anhydrous sodium sulfate and evaporation in vacuo gave a brown gum. This gum after passing through a neutral alumina column gave Δ^6-cholestene-3β,5α-diol (186) (0.17 g), crystallized from methanol, m.p. 146-150°C (lit.,[118] 147-150°C). ^{1}H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 14.

Mass spec. m/z (%): 402 (M^+, 1.1), 384 (31.2), 368 (11.3), 367 (16.7), 366 (49.8), 351 (16.6), 253 (13.1), 247 (32.8), 211 (10.7), 163 (17.4), 161 (10.1), 159 (25.8), 158 (27.0), 157 (24.7), 249 (32.9), 145 (37.6), 135 (70.3), 127 (24.1), 119 (50.5), 109 (39.9), 57 (100).

Reaction of 5β,6β-epoxycholestan-3β-ol (3) with LDEA:

A solution of 5β,6β-epoxycholestan-3β-ol (0.3 g) in ether (100 mL, dry) was refluxed gently for three days with lithium diethylamide prepared from n-butyllithium (9.33 mL, 1.6 M in hexane) and diethylamine

(1.6 mL) as described previously. Workup as usual afforded a mixture, which on addition of hexane and allowing to stand, gave Δ^4-cholesten-3β,6β-diol (190) (0.05 g), m.p. 253-255°C (lit.,[109] 258°C). ^{1}H NMR data are given in Table 4.

Mass spec. m/z (%): 402 (M^+, 2.2), 400 (8.8), 385 (19.5), 384 (61.8), 382 (10.6), 369 (33.9), 368 (25.1), 367 (20.9), 366 (39.0), 247 (37.7), 229 (35.6), 211 (22.1), 161 (32.2), 149 (37.7), 147 (39.5), 135 (69.0).

The mother liquor was chromatographed on neutral alumina. Elution with hexane and increasing concentrations of ether afforded first a compound more polar on silica gel tlc. Δ^6-Cholesten-3β,5α-diol (186) (0.03 g), crystallized from ethyl acetate, hexane, m.p. 182-185°C (lit.,[119] 181°C), identical with the authentic sample obtained in the previous experiment (tlc, ^{1}H NMR, ^{13}C NMR, mass spec.).

The less polar (on silica gel tlc) compound was highly retained in alumina column. It was eluted with ether-ethyl acetate (95:5) and was identified as Δ^6-cholesten-3β,5β-diol (189) (0.10 g), m.p. 138-140°C (lit.,[120] 139-140°C). ^{1}H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 14.

Mass spec. m/z (%): 402 (M^+, 0.5), 400 (0.4), 384 (7.0), 368 (5.6), 366 (11.6), 330 (28.6), 247 (12.8), 159 (10.4), 147 (10.3), 145 (15.0), 143 (14.4), 135 (22.5), 122 (16.2), 121 (17.3), 119 (20.1), 107 (17.3), 95 (25.0), 71 (86.2).

Reaction of 5α,6α-epoxycholestane (154) with LDEA:

The solution of 5α,6α-epoxycholestane (154) (0.5 g) in ether (50 mL, dry) was refluxed gently for one hour with lithium diethylamide prepared from n-butyllithium (5 mL, 2.4 M in hexane) and diethylamine (3.0 mL). The usual workup gave Δ^6-cholesten-5α-ol (187) (0.36 g), m.p. 86°C. ^{1}H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 14.

Mass spec. m/z (%): 386 (M^+, 33.9), 371 (24.9), 369 (25.9), 368 (78.3), 353 (24.6), 331 (13.8), 255 (39.6), 247 (71.5), 231 (20.3), 229 (11.3), 213 (26.7), 201 (18.6), 163 (32.6), 161 (40.1), 160 (34.1), 159 (48.2), 149 (37.3), 145 (86.5), 135 (54.1).

Exact mass, calculated for $C_{27}H_{46}O$ 386.348; observed 386.354.

Reaction of 5β,6β-epoxycholestane (155) with LDEA:

The solution of 5β,6β-epoxycholestane (155) (0.10 g) in ether (100 mL, dry) was refluxed gently for three days with lithium diethylamide prepared from n-butyllithium (3.1 mL, 1.6 M in hexane) and diethylamine (0.6 mL). Workup as usual afforded a crude product, the ^{1}H NMR of which showed the formation of 5 to 10% of Δ^4-cholesten-6β-ol (193),[103] only the starting epoxide was re-isolated. ^{1}H NMR data are given in Table 4.

Reaction of 5α,6α-epoxycholestan-3α-ol (125) with LDEA:

The solution of 5α,6α-epoxycholestan-3α-ol (125) (0.2 g) in ether (100 mL, dry) was refluxed gently for three days with the lithium diethylamide prepared *in situ* from n-butyllithium (6.1 mL, 1.6 M in hexane) and diethylamine (1.1 mL) as described previously. The usual workup afforded a gummy mixture which on crystallization from ethyl acetate-hexane gave Δ^4-cholesten-3α,6α-diol (194) (0.035 g), m.p. 191-193°C (lit.,[121] 189-191°C). ^{1}H NMR data are presented in Table 4, and ^{13}C NMR data are presented in Table 15.

Mass spec. m/z (%): 402 (M^+, 0.8), 400 (4.4), 384 (74.3), 369 (68.0), 368 (10.9), 367 (15.4), 366 (25.6), 356 (16.1), 275 (18.3), 271 (24.5), 247 (31.2), 229 (54.6), 211 (21.2), 161 (30.8), 159 (32.7), 135 (73.5), 123 (50.1), 121 (54.5), 107 (69.5).

The mother liquor left after crystallization was chromatographed on silica gel (100 g). Elution with chloroform-ethyl acetate (95:5) gave the starting material, 5α,6α-epoxycholestan-3α-ol (0.05 g), which was found to be identical with the authentic sample (tlc, ^{1}H NMR, mass spec). Further elution with chloroform-ethyl acetate (85:15) gave another product, crystallized from hexane, Δ^6-cholesten-5α,3α-diol (195) (0.035 g), m.p. 146-149°C. ^{1}H NMR data are given in Table 4.

Mass spec. m/z (%): 402 (M^+, 9.7), 385 (12.2), 384 (31.6), 369 (25.4), 368 (18.9), 367 (22.0), 366 (62.7), 351 (25.6), 271 (11.1), 253 (18.6), 247 (50.2), 245 (13.8), 229 (19.3), 215 (10.9), 211 (20.3), 206 (10.3), 191 (23.9), 175 (26.0), 163 (32.1), 159 (41.7), 149 (58.7).

Exact mass, calculated for $C_{27}H_{46}O_2$, 402.349; observed, 402.346.

Reaction of 5β,6β-epoxycholestan-3α-ol (126) with LDEA:

A solution of 5β,6β-epoxycholestan-3α-ol (126) (0.1 g) in ether (100 mL, dry) was refluxed gently with lithium diethylamide, prepared from n-butyllithium (3.10 mL, 1.6 M in hexane) and diethyl amine (0.6 mL), for three days, as described previously. Workup as usual afforded only starting material (0.75 g), identical with the authentic sample (m.p., tlc, ^{1}H NMR, mass spec).

Reaction of 5β,6β-epoxycholestan-3,3-ethylene dioxyketal (151) with LDEA:

A solution of 5β,6β-epoxycholestan-3,3-ethylene dioxyketal (151) (0.50 g) in ether (150 mL, dry) was gently refluxed with diethylamide, prepared from n-butyllithium (140 mL, 1.6 M in hexane) and diethylamine (3.2 mL), for three days as described previously. Workup as usual afforded a mixture which was chromatographed on silica gel. Elution with chloroform-ethyl acetate (90:10) gave Δ^4-cholesten-3-one-6β-ol (177) (0.130 g), crystallized from petroleum ether (60-100°C), m.p., 195-196°C (lit. [116] m.p. 194°C). ^{1}H NMR data are given in Table 4, and ^{13}C NMR data are presented in Table 10.

Mass spec. m/z (%): 400 (M^+, 65.8), 398 (22.9), 385 (16.8), 384 (16.9), 383 (12.3), 382 (15.9), 380 (14.4), 387 (19.5), 385 (12.5), 369 (12.2),

361 (12.6), 247 (37.0), 246 (26.4), 245 (49.2), 229 (23.4), 175 (23.0). A total of 0.2 g of compound of very low solubility, and high melting point (above 300°C) was obtained, but not investigated further.

Reaction of 5α,6α-epoxycholestan-3,3-ethylene dioxyketal (150) with LDEA:

A solution of 5α,6α-epoxycholestane-3,3-ethylene dioxyketal (150) (0.20 g) in ether (50 mL, dry) was gently refluxed with lithium diethylamide, prepared from n-butyllithium (3.0 mL, 1.6 M in hexane) and diethylamine (0.6 mL), for three days as described previously. Workup as usual, avoiding acid treatment, gave an oil; the proton NMR and mass spectrom of this showed the presence of Δ^4-cholesten-6α-ol-3,3-ethylene dioxyketal (173) which on usual workup with acid and column chromatography on silica gel gave Δ^4-cholesten-3-one-6α-ol (174) (0.110 g) identical with the authentic sample obtained previously (m.p., tlc, ^{1}H NMR, ^{13}C NMR, mass spec.). During workup, addition of water precipitated a white insoluble material which was not identified.

Reaction of 4β,5β-epoxycholestan-3β-ol (164) with LDEA:

A solution of 4β,5β-epoxycholestan-3β-ol (164) in ether (100 mL, 1 mL THF, dry) was gently refluxed under a dry nitrogen setup, with lithium diethylamide, prepared from n-butyllithium (6.2 mL, 1.6 M in hexane) and diethylamine (1.14 mL), for three days as described previously. Workup as usual afforded only starting material (0.17 g) identical with the authentic sample (tlc, m.p., ^{1}H NMR, mass spec.).

Reaction of 4β,5β-epoxycholestan-3α-ol (169) with LDEA:

A solution of 4β,5β-epoxycholestan-3α-ol (169) (0.10 g) dissolved in tetrahydrofuran (5 mL, dry) and ether (75 mL, dry) was gently refluxed under a dry nitrogen setup (as described previously) with lithium diethylamide, prepared from n-butyllithium (3.1 mL, 1.6 M in hexane) and diethylamine (0.6 mL), for three days. Workup as usual afforded only starting material (0.087 g), identical with the authentic sample (m.p., tlc, ^{1}H NMR, mass spec.).

Reaction of 4α,5α-epoxycholestane (160) with LDEA:

A solution of 4α,5α-epoxycholestane (160) (0.1 g) in ether (100 mL, dry) was refluxed gently, under dry nitrogen (as described previsouly) with lithium diethylamide, prepared from n-butyllithium (3.1 mL, 1.6 M in hexane) for three days. Workup as usual afforded a crude mixture which was chromatographed on neutral alumina. Elution with hexane-ether (90:10) gave Δ^3-cholesten-5α-ol (203) (0.04 g) crystallized from aqueous acetone, m.p. 79-80° (lit. [122] m.p. 75-76°C). ^{1}H NMR data are presented in Table 4, and ^{13}C NMR data are given in Table 15.

Mass spec. m/z (%): 386 (M^+, 4.1), 368 (100), 354 (10.4), 353 (34.9), 332 (57.8), 314 (10.5), 255 (35.5), 247 (26.4), 213 (40.7), 161 (26.5), 160 (24.1), 159 (30.8), 147 (58.4), 145 (45.2), 135 (37.9), 118 (50.6).

Further elution with increasing ether concentration gave the more polar compound. Being slightly impure, it was chromatographed on preparative tlc, which yielded Δ^5-cholesten-5α-ol (204) (0.015 g) crystallized from acetone-pentane, m.p., 140-142°C (lit.,[123] 144-145°C). ^{1}H NMR data are given in Table 4.

Mass spec. m/z (%): 386 (M^+, 38.8), 371 (13.4), 370 (13.4), 369 (23.5), 368 (71.4), 353 (27.1), 332 (10.6), 255 (31.1), 247 (25.4), 213 (20.7), 163 (20.7), 161 (30.2), 159 (23.4), 149 (32.3), 147 (58.6), 145 (38.6), 81 (100).

Reaction of 4β,5β-epoxycholestane (161) with LDEA:

A solution of 4β,5β-epoxycholestane (161) (0.1 g) in ether (50 mL) was refluxed gently under a dry nitrogen atmosphere, with lithium diethylamide, prepared from n-butyllithium (3.1 mL, 1.6 M in hexane) and diethylamine (0.6 mL) as described previously, for three days. Workup as usual afforded a mixture which was chromatographed on neutral alumina to remove brownish material. The mixture was separated by preparative layer chromatography; the plates were developed in hexane-ether (50:50), and two elutions gave a reasonable separation. The compounds with higher R_f value was identified as Δ^5-cholesten-4β-ol (200) (0.030 g), crystallized from aqueous methanol, m.p. 128-130°C (lit.,[124] 131-132°C). ^{1}H NMR data are presented in Table 4.

Mass spec. m/z (%): 386 (M^+, 6.2), 369 (26.4), 368 (8.9), 353 (25.9), 332 (53.3), 255 (23.3), 213 (31.7), 191 (60.1), 178 (40.0), 175 (47.1), 163 (39.0), 161 (23.1), 159 (22.3), 149 (41.1), 147 (43.1), 135 (37.8), 109 (43.6), 107 (42.6), 106 (55.9), 57 (100).

The compound with lower R_f value was identified as Δ^3-cholesten-5β-ol (199) (0.06 g), crystallized from methanol (aqueous), m.p. 90-92°C (lit.,[123] 89-91°C). ^{1}H NMR data are given in Table 4, and ^{13}C data are presented in Table 15.

Mass spec. m/z (%): 386 (M^+, 53.8), 368 (53.9), 353 (22.1), 255 (24.5), 213 (25.6), 161 (20.9), 149 (33.3), 147 (45.5), 145 (25.3), 137 (26.4), 135 (31.1), 124 (67.3), 123 (43.3), 121 (36.1), 109 (85.1), 107 (60.4), 95 (79.1), 55 (100).

Reaction of 4α,5α-epoxycholestan-3β-ol (167) with LDEA:

A solution of 4α,5α-epoxycholestan-3β-ol (167) (0.10 g) in ether (75 mL, dry) was gently refluxed under conditions described previously, with lithium diethylamide, prepared from n-butyllithium (3.2 mL, 1.6 M in hexane) and diethylamine (0.6 mL), for three days. Workup as usual afforded a mixture which was chromatographed on neutral alumina. Elution with hexane-ether (90:10) gave starting material, 4α,5α-epoxycholestan-3β-ol (0.050 g), crystallized from methanol, identical with the authentic sample (m.p., tlc, ^{1}H NMR, mass spec.). Further elution with increasing polarity of solvent system afforded Δ^5-cholesten-3β,4α-diol (202) (0.01 g) crystallized from methanol (aqueous), m.p. 216-219°C (lit.,[124] 223°C from acetone). ^{1}H NMR data are presented in Table 4.

Mass spec. m/z (%): 402 (M^+, 1.2), 384 (8.6), 332 (66.8), 149 (22.8), 147 (25.8), 135 (27.5), 133 (21.5), 109 (27.9), 100 (48.5), 82 (73.0), 55 (100).

Reduction of Cholestenone (148) with Aluminum isopropoxide[299]

Cholestenone (10.0 g) was dissolved in 200 mL of absolute isopropyl alcohol. To this was added a solution of 20 g of aluminum isopropoxide in

150 mL of absolute isopropyl alcohol. The solution was refluxed at such a rate that slow distillation occurred; the reaction was continued for 10 hours. The solution was cooled in ice, and 100 mL of ether and a sufficient quantity of cold 20% potassium hydroxide solution were added to produce two clear layers. The acqueous layer was removed, and the ethereal layer was washed repeatedly with water and dried over anhydrous sodium sulfate. The ethereal extract was evaporated *in vacuo*. The crystalline residue was dissolved in 100 mL of boiling ether. Then an equal volume of methanol was added, and the solution was cooled in ice salt mixture. The crystalline material was filtered and dried, yielding 7.0 g of crude product consisting of 1:1 mixture of Δ^4-cholesten-3α-ol (170) and Δ^4-cholesten-3β-ol (163), m.p. 138-140°C (lit.,[299] 141°C).

Isolation of Δ^4-cholesten-3α-ol (170):

7.0 g of the above crude reaction product were dissolved in 150 mL of absolute ethanol and treated with 6.0 g of digitonin in 50 mL of 95% ethanol. After 24 hours, the precipitate was filtered off and the filtrate was evaporated to dryness *in vacuo* at 30-35°C. The residue was repeatedly extracted with ether. The combined ethereal extract was evaporated to dryness *in vacuo*. Crystallization from acetone water yielded the product still contaminated slightly by β-isomer. Therefore, it was chromatographed on silica gel. Elution with benzene-ether afforded pure Δ^4-cholesten-3α-ol (170). Crystallization from acetone-water gave sample (3.3 g) having m.p. 84°C (lit.,[299] 84°C). ^{1}H NMR data are given in Table 1, and ^{13}C NMR data are presented in Table 12.

Mass spec. m/z (%): 386 ($M^{+\cdot}$, 2.8), 371 (2.3), 368 (89.2), 353 (26.1), 316 (6.2), 261 (19.5), 255 (33.2), 247 (25.3), 229 (9.2), 213 (24.8), 161 (27.5), 160 (24.7), 159 (25.7), 147 (77.0), 145 (53.1).

4α,5α-Epoxycholestan-3α-ol (171)

Δ^4-Cholesten-3α-ol (170) (0.8 g) was dissolved in methylene chloride (20 mL) and the solution was cooled to 0°C. A solution of m-chloroperbenzoic acid (0.9 g) in methylene chloride (20 mL) was added dropwise to the above solution at 0°C and the reaction mixture was allowed to stand overnight at 0°C. Workup as usual afforded 4α,5α-epoxycholestan-3α-ol (171) (0.5 g). It was isolated as a gum.[299] ^{1}H NMR data are given in Table 3, and ^{13}C NMR data are presented in Table 11.

Mass spec. m/z (%): 402 ($M^{+\cdot}$, 5.9), 400 (4.1), 384 (8.7), 369 (4.6), 368 (3.5), 333 (24.4), 332 (100), 317 (15.5), 247 (15.8), 229 (11.0), 219 (10.0), 201 (18.0), 174 (15.0), 147 (26.1), 135 (20.6), 133 (17.9), 123 (19.9), 121 (26.7), 109 (3.10), 107 (32.0), 105 (28.2), 95 (45.0).

Table 1. ^{1}H NMR data

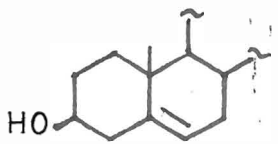
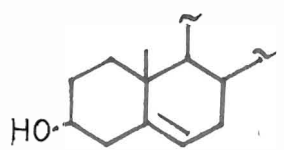
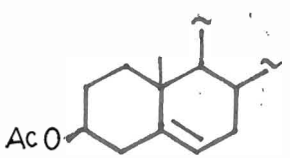
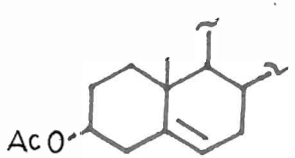
Compound	C3	C4	C6	C18	C19	extra
 (1)	3.19-3.28 (c m ,H_α)	--	5.35 (d, 1H, olefinic $J_{5.0\ Hz}$)	0.68 (s, 3H)	0.95 (s, 3H)	--
 (121)	4.03 (c m, 1H_β)	--	5.45 (d, 1H, olefinic, $J_{5.4\ Hz}$)	0.68 (s, 3H)	1.1 (s, 3H)	3.45 (br s, 1H, OH)
 (116)	4.20-4.90 (br m, 1H_α)	--	5.40 (d, 1H, olefinic, $J_{5.5\ Hz}$)	0.68 (s, 3H)	1.04 (s, 3H)	2.02 (s, 3H, OAc)
 (127)	5.0 (c m, 1H_β)	--	5.38 (d, 1H, olefinic $J_{5.0\ Hz}$)	0.68 (s, 3H)	1.0 (s, 3H)	2.0 (s, 3H, OAc)

Table 1 (continued)

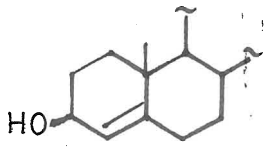
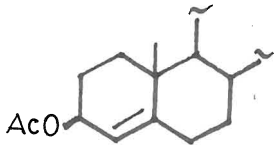
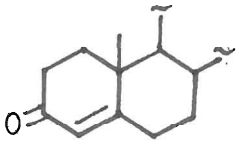
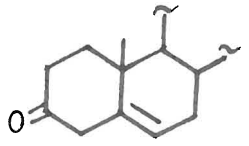
Compound	C3	C4	C6	C18	C19	extra
 (163)	3.9-4.4 (c m, 1H$_\alpha$)	5.32 (br s, 1H olefinic)	--	0.69 (s, 3H)	1.07 (s, 3H)	--
 (165)	5.10-5.52 (m, 2H, C3 H$_\alpha$, C4 H olefinic)		--	0.70 (s, 3H)	1.09 (s, 3H)	2.06 (s, 3H, OAc)
 (148)	--	5.72 (s, 1H, olefinic)	--	0.70 (s, 3H)	1.15 (s, 3H)	--
 (122)	--	2.90, 3.18 (d(AB), 2H J_{gem}16.0 Hz)	5.30 (d, 1H olefinic J6.0 Hz)	0.68 (s, 3H)	1.07 (s, 3H)	--

Table 1 (continued)

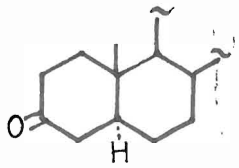
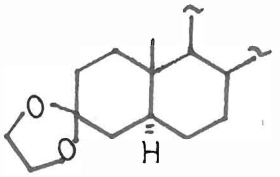
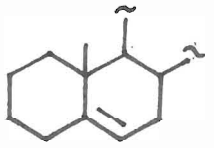
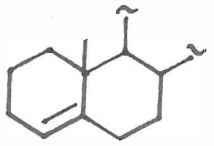
Compound	C3	C4	C6	C18	C19	extra
 (180)	--	--	--	0.66 (s, 3H)	1.0 (s, 3H)	--
 (181)	--	--	--	0.67 (s, 3H)	0.91 (s, 3H)	--
 (153)	--	--	5.21 (d, 1H, olefinic $J_{3.0\ Hz}$)	0.68 (s, 3H)	1.0 (s, 3H)	--
 (159)	--	5.21-5.46 (br t, 1H, olefinic)	--	0.67 (s, 3H)	1.03 (s, 3H)	--

Table 1 (continued)

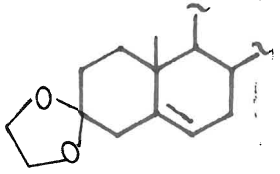
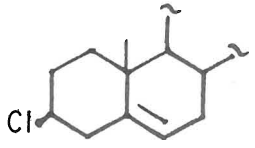
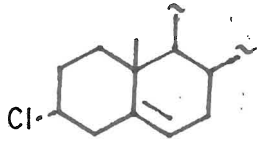
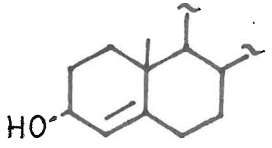
Compound	C3	C4	C6	C18	C19	extra
 (149)	--	--	5.35 (d, 1H, olefinic $J_{5.0\ Hz}$)	0.68 (s, 3H)	1.30 (s, 3H)	3.85 (s, 4H, -O-CH -CH -O-)
 (152)	3.45-4.0 (c m, $1H_{\alpha}$)	--	5.38 (d, 1H, olefinic, $J_{5.0\ Hz}$)	0.70 (s, 3H)	1.02 (s, 3H)	--
 (158)	4.0-4.65 (c m, $1H_{\beta}$)	--	5.40 (d, 1H, olefinic $J_{4.0\ Hz}$)	0.65 (s, 3H)	1.02 (s, 3H)	--
 (170)	4.20 (br s, $1H_{\beta}$)	5.34 (d, 1H, olefinic $J_{4.0\ Hz}$)	--	0.74 (s, 3H)	--	--

Table 2. ^{1}H NMR

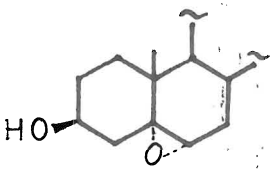
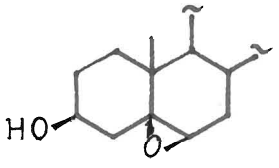
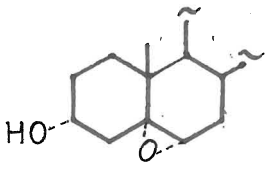
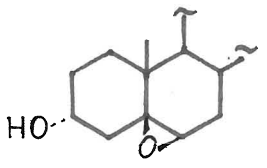
Compound	C3	C6	C18	C19	extra
 (2)	3.5-3.95 (c m, 1H$_\alpha$)	2.90 (d, 1H$_\beta$, J 3.8 Hz)	0.68 (s, 3H)	1.03 (s, 3H)	--
 (3)	3.32-4.0 (br m, 1H$_\alpha$)	3.07 (d, 1H$_\alpha$, J 2.4 Hz)	0.62 (s, 3H)	1.0 (s, 3H)	--
 (125)	4.09 (m, 1H$_\beta$, $W_{1/2}$ 7.0 Hz)	2.87 (d, 1H$_\beta$, J 4.0 Hz)	0.65 (s, 3H)	1.02 (s, 3H)	--
 (126)	4.21 (br s, 1H$_\beta$)	3.10 (d, 1H$_\alpha$, J 4.0 Hz)	0.64 (s, 3H)	1.0 (s, 3H)	--

Table 2 (continued)

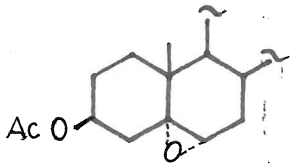
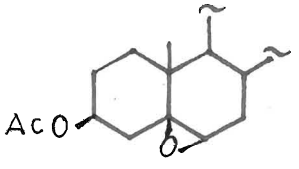
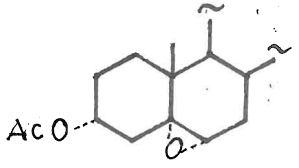
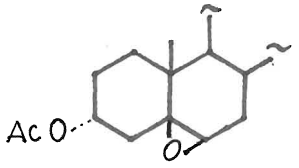
Compound	C3	C6	C18	C19	extra
 (2A)	4.55-5.3 (br m, 1H$_\alpha$)	2.90 (d, 1H$_\beta$, J 4.0 Hz)	0.65 (s, 3H)	1.08 (s, 3H)	2.0 (s, 3H, OAc)
 (3A)	4.30-5.18 (br m, 1H$_\alpha$)	3.07 (d, 1H$_\alpha$, J 2.4 Hz)	0.65 (s, 3H)	1.02 (s, 3H)	2.05 (s, 3H, OAc)
 (129)	5.12 (br s, 1H$_\beta$, $W_{\frac{1}{2}}$ 5.0 Hz)	2.78 (d, 1H$_\beta$, J 4.0 Hz)	0.65 (s, 3H)	1.10 (s, 3H)	2.07 (s, 3H, OAc)
 (130)	5.02-5.25 (c m, 1H$_\beta$)	3.03 (d, 1H$_\alpha$, J 3.0 Hz)	0.67 (s, 3H)	1.02 (s, 3H)	2.08 (s, 3H, OAc)

Table 2 (continued)

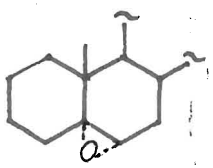
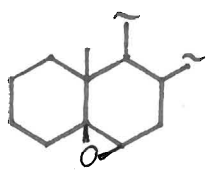
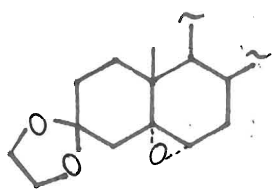
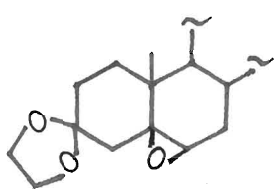
Compound	C3	C6	C18	C19	extra
 (154)	--	2.88 (d, 1H$_\beta$, J 4.0 Hz)	0.65 (s, 3H)	1.02 (s, 3H)	--
 (155)	--	3.0 (d, 1H$_\alpha$, J 2.0 Hz)	0.65 (s, 3H)	0.98 (s, 3H)	--
 (150)	--	2.83 (d, 1H$_\beta$, J 2.1 Hz)	0.61 (s, 3H)	1.03 (s, 3H)	3.95 (s, 4H, -O-CH_2-CH_2-O-)
 (151)	--	3.03 (d, 1H$_\alpha$, J 2.1 Hz)	0.61 (s, 3H)	1.0 (s, 3H)	3.90 (s, 4H, -O-CH_2-CH_2-O-

Table 3. ^{1}H NMR data

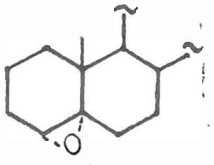
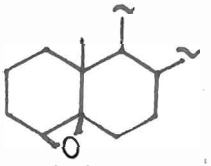
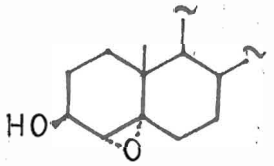
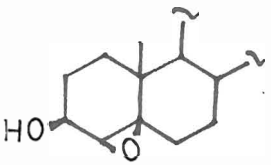
Compound	C3	C4	C18	C19	extra
 (160)	--	2.93 (m, 1H$_\beta$ $W_{1/2}$ 6.0 Hz)	0.65 (s, 3H)	1.07 (s, 3H)	--
 (161)	--	2.98 (m, 1H$_\alpha$, $W_{1/2}$ 6.0 Hz)	0.65 (s, 3H)	1.02 (s, 3H)	--
 (167)	3.70-4.27 (c m, 1H$_\alpha$)	2.92 (s, 1H$_\beta$)	0.68 (s, 3H)	1.11 (s, 3H)	--
 (164)	3.95-4.25 (c m, 1H$_\alpha$)	3.18 (d, 1H$_\alpha$, $J_{4.0\ Hz}$)	0.67 (s, 3H)	1.01 (s, 3H)	--

Table 3 (continued)

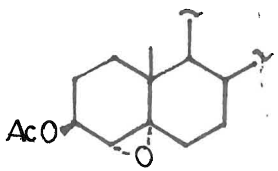
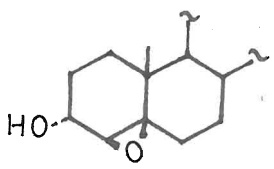
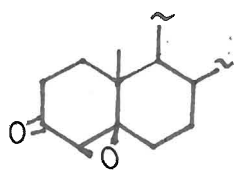
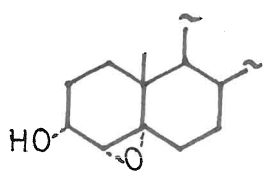
Compound	C3	C4	C18	C19	extra
 (166)	4.60-5.33 (br m, $1H_\alpha$)	2.98 (s, $1H_\beta$)	0.70 (s, 3H)	1.20 (s, 3H)	2.10 (s, 3H, OAc)
 (169)	4.02-4.22 (c m, $1H_\beta$)	2.89 (s, $1H_\alpha$)	0.68 (s, 3H)	1.03 (s, 3H)	--
 (168)	--	3.04 (s, $1H_\alpha$)	0.72 (s, 3H)	1.08 (s, 3H)	--
 (171)	4.0 (m, $1H_\beta$)	3.19 (d, $1H_\beta$, $J_{4.0\ Hz}$)	0.71 (s, 3H)	--	--

Table 4. ^{1}H NMR data

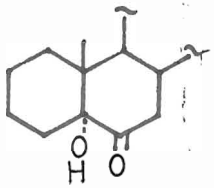
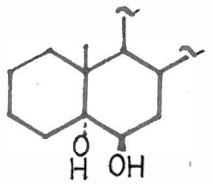
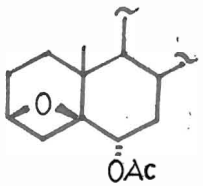
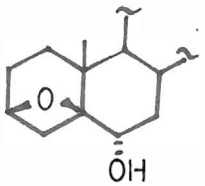
Compound	C3	C4	C6	C18	C19	extra
 (157)	--	--	--	0.63 (s, 3H)	0.95 (s, 3H)	--
 (156)*	--	--	3.49 (br s, 1H$_\alpha$)	0.67 (s, 3H)	1.58 (s, 3H)	2.17 (m, 1H, OH) 1.97 (d, 1H, OH)
 (131)	4.20 (br s, 1H$_\alpha$)	2.15 (d d, 1H$_\beta$ J_{gem} 16.0 Hz J_{vic} 4.0 Hz) 2.85 (d, 1H$_\alpha$, J_{gem} 16.0 Hz)	4.65 (br s, 1H$_\beta$)	0.66 (s, 3H)	1.13 (s, 3H)	2.0 (s, 3H, OAc)
 (134)	4.10-4.41 (c m, 1H$_\alpha$)		3.50-3.70 (c m, 1H$_\beta$)	0.68 (s, 3H)	1.12 (s, 3H)	3.10 (br s, 1H, OH)

Table 4 (continued)

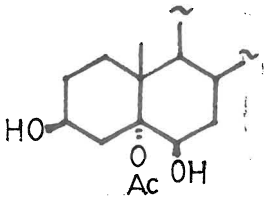
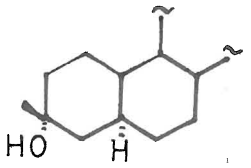
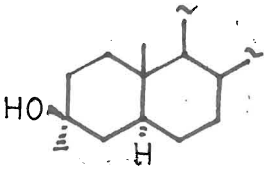
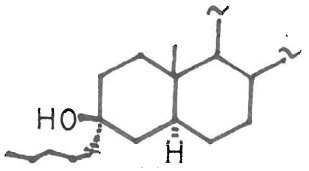
Compound	C3	C4	C6	C18	C19	extra
 (132)	3.14-3.70 (br m, $1H_\alpha$)	--	4.45-4.70 (c m, $1H_\alpha$)	0.63 (s, 3H)	--	1.98 (s, 3H, OAc)
 (185)	--	--	--	0.65 (s, 3H)	1.2 (s, 3H)	--
 (184)	--	--	--	0.65 (s, 3H)	--	--
 (183)	--	--	--	0.65 (s, 3H)	--	--

Table 4. (continued)

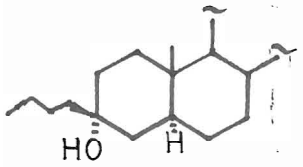
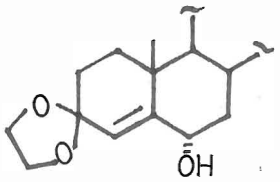
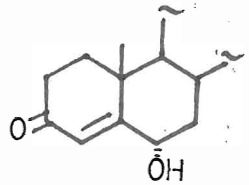
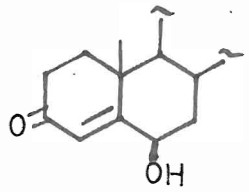
Compound	C3	C4	C6	C7	C18	C19	extra
 (182)	--	--	--	--	0.66 (s, 3H)	0.91 (s, 3H)	--
 (173)	--	5.67 (s, 1H, olefinic)	4.20 (m, $1H_\beta$)	--	0.68 (s, 3H)	1.01 (s, 3H)	4.01 (s, 4H, $O-CH_2-CH_2-O$)
 (174)	--	6.18 (s, 1H, olefinic)	4.28 (m, $1H_\beta$ $W_{1/2}$ 20 Hz)	--	0.70 (s, 3H)	1.17 (s, 3H)	--
 (177)	--	5.78 (s, 1H, olefinic)	4.21 (m, $1H_\alpha$ $W_{1/2}$ 6.8 Hz)	--	0.68 (s, 3H)	1.30 (s, 3H)	--

Table 4 (continued)

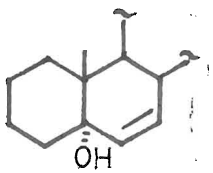
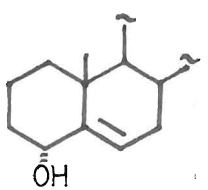
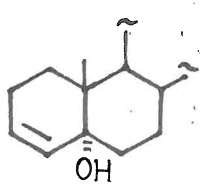
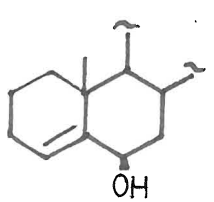
Compound	C3	C4	C6	C7	C18	C19	extra
 (187)	--	--	5.60 (s, 3H, C6, olefinic)	5.60 C7	0.68 (s, 3H)	1.04 (s, 3H)	2.95 (br s, 1H, OH)
 (204)	--	3.66-4.30 (m, $1H_\beta$)	5.30-6.0 (m, 1H, olefinic)	--	0.64 (s, 3H)	1.0 (s, 3H)	--
 (203)	5.60-5.78 (c m, 2H, olefinic)	5.60-5.78 C3, C4	--	--	0.68 (s, 3H)	0.92 (s, 3H)	--
 (193)	--	5.6 (m, 1H, olefinic)	4.21 (m, $1H_\alpha$)	--	--	--	--

Table 4 (continued)

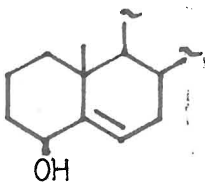
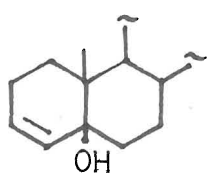
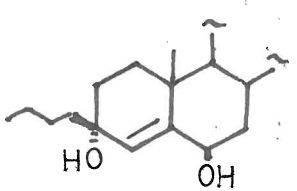
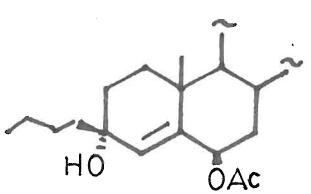
Compound	C3	C4	C6	C7	C18	C19	extra
 (200)	--	3.33-4.35 (br m, $1H_\alpha$)	5.63 (br s, olefinic)	--	0.66 (s, 3H)	1.20 (s, 3H)	--
 (199)	5.73 (t, d(AB), 1H olefinic, $J_{3,4}$ = 3.0 Hz, $J_{3,2}$ = 1.0 Hz)	5.40 (d(AB), 1H, olefinic $J_{3,4}$ = 5.0 Hz)	--	--	0.66 (s, 3H)	0.99 (s, 3H)	3.56 (m, 1H, OH)
 (175)	--	5.44 (s, 1H, oleinfic)	4.21 (m, $1H_\alpha$, $W_{1/2}$ 6.0 Hz)	--	0.70 (s, 3H)	1.27 (s, 3H)	--
 (175A)	--	5.60 (s, 1H, olefinic)	5.20-5.45 (br s, $1H_\alpha$)	--	0.70 (s, 3H)	1.26 (s, 3H)	2.0 (s, 3H, OAc)

Table 4 (continued)

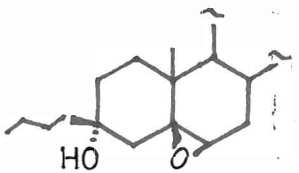
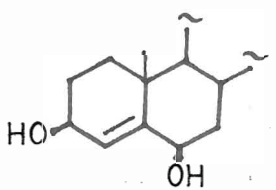
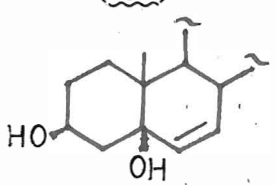
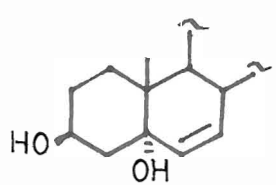
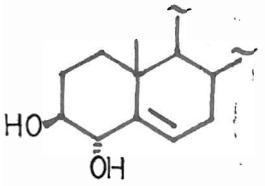
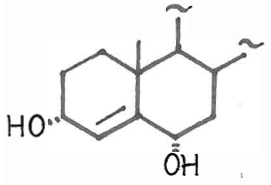
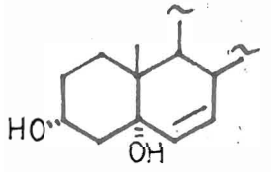
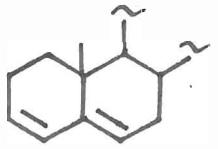
Compound	C3	C4	C6	C7	C18	C19	extra
 (176)*	--	--	3.07 (d, $1H_\alpha$, J = 2.2 Hz)	--	0.65 (s, 3H)	0.97 (s, 3H)	--
 (190)	3.5-3.85 (c m, $1H_\alpha$)	5.65 (br s, 1H, olefinic)	4.10-4.45 (c m, $1H_\alpha$)	--	0.68 (s, 3H)	--	--
 (189)	3.93-4.27 (c m, $1H_\alpha$)	--	5.50 (s, 3H, C6, C7 olefinic)	5.50	0.70	--	3.19 (m, 1H, OH)
 (186)	3.84-4.21 (br m, $1H_\alpha$)	--	5.58 (s, 3H, C6, C7, olefinic)	5.58	0.67 (s, 3H)	--	--

Table 4 (continued)

Compound	C3	C4	C6	C7	C18	C19	extra
 (202)	3.70 (m, $1H_\alpha$)	4.0-4.90 (br m, $1H_\beta$)	5.51 (m, 1H, olefinic)	--	0.67 (s, 3H)	--	--
 (194)	3.76-4.1 (c m, 2H, C3, C6)	5.29 (br s, 1H)	3.76-4.1 (c m, 2H, C3, C6)	--	0.69 (s, 3H)	1.08 (s, 3H)	--
 (195)	4.01-4.21 (m, $1H_\beta$)	--	5.67 (s, 2H, C6, C7, olefinic)	5.67	0.75 (s, 3H)	1.30 (s, 3H)	--
 (172)	5.3-5.75 (c m, 2H, C3, C6, olefinic)	5.8-6.7 (d, 1H, olefinic, J_{AB} = 10.0 Hz)	5.3-5.75 (c m, 2H, C3, C6, olefinic)		0.7 (s, 3H)	--	--

* spectra recorded at 400 MHz. c = complex, m = multiplet, br = broad, s = singlet, d = doublet

Table 5. ^{13}C NMR data

Carbon number	(1)[293]	(121)	(127)	(125)	(126)
1	37.3	33.3	33.5	28.7	33.2
2	31.6	29.0	26.2	29.0	28.5
3	71.6	67.1	70.7	68.0	67.2
4	42.2	39.9	36.3	36.5	40.0
5	140.6	138.7	138.5	65.5	62.2
6	121.4	124.0	122.1	57.8	63.7
7	31.9	32.0	31.9	28.1	32.5
8	31.9	32.0	31.9	29.8	29.9
9	50.2	50.5	50.0	42.8	50.5
10	36.5	37.4	37.1	35.7	35.6
11	21.1	20.8	20.8	20.4	21.7
12	39.8	39.9	39.9	39.6	40.0
13	42.3	42.2	42.4	42.4	42.4
14	56.8	56.8	56.8	57.0	56.4
15	24.3	24.3	24.3	24.1	24.2
16	28.3	28.2	28.2	28.7	28.2
17	56.2	56.2	56.3	56.0	56.3
18	12.0	11.9	11.9	11.9	11.8
19	19.5	18.7	18.9	15.4	17.0
20	35.8	35.8	35.8	35.8	35.8
21	18.8	18.7	18.7	18.7	18.7
22	36.2	36.3	36.3	36.3	36.2
23	23.9	23.9	23.9	23.9	23.9
24	39.5	39.6	39.6	39.6	39.6
25	28.0	28.1	28.0	28.1	28.1
26	22.6	22.6	22.6	22.6	22.6
27	22.8	22.6	22.8	22.8	22.8
acetate			170.8 21.4		

Table 6. ^{13}C NMR data

Carbon number	(129)	(130)	(2A)	(3A)
1	28.0	33.8	32.2	36.7
2	26.0	25.5	27.3	27.3
3	69.6	70.5	71.4	71.4
4	33.8	36.7	36.2	38.0
5	63.5	61.7	56.1	62.6
6	56.7†	63.3	59.2	63.6
7	28.4	32.5	28.0	32.6
8	29.7	29.8	29.9	29.8
9	42.4	50.3	42.5	51.0
10	35.4	35.5	35.1	35.1
11	20.4	21.7	20.6	21.3
12	39.5	39.9	39.5	39.6
13	42.4	42.4	42.4	42.4
14	57.0†	56.4	56.8	56.3
15	24.0	24.2	24.0	24.2
16	28.7	28.2	28.8	28.1
17	56.0	56.4	55.9	56.3
18	11.9	11.8	11.9	11.8
19	15.6	17.2	15.9	17.0
20	35.8	35.8	35.8	35.8
21	18.7	18.7	18.7	18.7
22	36.2	36.2	36.2	36.2
23	23.9	23.9	23.9	23.9
24	39.5	39.6	39.5	39.6
25	28.0	28.2	28.0	28.1
26	22.5	22.6	22.6	22.6
27	22.8	22.8	22.8	22.8
acetate	170.1	172.0	170.2	170.6
	21.5	21.3	21.3	21.3

* chemical shifts may be interchanged

† distinguished by decoupling

Table 7. ^{13}C NMR data

Carbon number	(2)	(3)	(149)	(150)	(151)
1	32.5	37.2	36.3	31.4	36.2
2	31.0	31.0	31.1	31.0	30.9
3	68.5	69.4	109.6	109.0	109.6
4	39.9	42.2	41.8	39.2	41.6
5	66.0	63.0	140.2	64.1	63.0
6	59.5	63.7	122.2	57.5	63.3
7	28.1	32.7	31.8	28.0	32.4
8	29.9	29.8	32.0	29.9	29.8
9	42.6	51.4	49.8	42.2	49.9
10	34.9	34.9	36.7	35.1	35.1
11	20.6	22.0	21.1	20.6	22.0
12	39.9	39.0	39.9	39.6	39.9
13	42.4	42.3	42.4	42.4	42.4
14	56.9	56.3	56.8	57.0	56.9
15	24.1	24.2	24.3	24.0	24.2
16	28.8	28.2	28.2	28.6	28.2
17	55.9	56.3	56.2	55.9	56.4
18	11.9	11.8	11.9	11.9	11.8
19	15.9	17.0	18.9	15.6	17.1
20	35.8	35.8	35.8	35.8	35.7
21	18.7	18.7	18.7	18.7	18.7
22	36.2	36.2	36.3	36.2	39.6
23	23.9	23.9	23.9	23.9	23.9
24	39.6	39.6	39.6	39.6	39.6
25	28.1	28.0	28.1	28.0	28.0
26	22.6	22.6	22.6	22.6	22.6
27	22.8	22.8	22.8	22.8	22.8
others			64.5	64.6	64.3
			64.2	64.1	64.2

Table 8. ^{13}C NMR data

Carbon number	(154)	(155)	(160)	(161)
1	34.5	35.8	31.3	23.9
2	21.8	21.7	22.5	19.3
3	23.4	28.1	28.5	29.8
4	31.0	32.9	60.8	61.5
5	66.1	65.8	66.1	65.7
6	60.3	63.7	30.4	30.7
7	28.1	32.9	28.5	31.6
8	29.9	30.1	35.7*	35.2
9	43.0	51.7	50.4	46.6
10	36.3	36.0	35.9*	35.2
11	20.4	21.5	20.8	21.4
12	39.6	39.6	40.0	40.0
13	42.4	42.4	42.8	42.7
14	57.1	56.5	56.0**	56.4
15	24.0	24.2	24.3	24.3
16	29.3	28.1	28.3	28.3
17	56.1	56.4	56.3**	56.4
18	11.9	11.8	12.2	12.1
19	15.9	17.2	16.0	17.4
20	35.8	35.8	35.9*	35.9
21	18.7	18.7	18.7	18.7
22	36.3	36.2	36.3	36.3
23	23.9	23.9	24.0	23.9
24	39.6	39.6	39.6	39.6
25	28.1	28.1	28.1	28.1
26	22.6	22.6	22.6	22.6
27	22.8	22.8	22.9	22.9

* chemical shifts may be interchanged

** chemical shifts may be interchanged

Table 9. ^{13}C NMR data

Carbon number	(175)	(175A)	(182)	(183)
1	37.0	37.0	33.2	36.4
2	32.8	32.7	35.9	37.3
3	71.6	71.4	71.6	73.0
4	131.5	134.4	44.4	43.9
5	146.3	141.3	41.0	42.7
6	74.4	76.0	28.7	28.8
7	39.2	42.7	32.1	32.2
8	30.3	31.0	34.0	34.6
9	54.2	54.0	54.3	54.7
10	36.8	36.2	35.7	35.7
11	21.1	21.1	21.1	21.4
12	39.9	39.9	40.1	40.2
13	42.7	42.7	42.8	42.8
14	56.4	56.1	56.6	56.6
15	24.2	24.2	24.3	24.3
16	28.4	28.2	28.3	28.1
17	56.3	56.3	56.4	56.6
18	12.0	12.1	12.1	12.2
19	21.3	20.6	11.2	12.2
20	35.8	35.8	35.9	35.9
21	18.7	18.7	18.7	18.7
22	36.2	36.2	36.3	36.3
23	23.9	23.9	23.9	24.0
24	39.6	39.6	39.6	39.6
25	28.1	28.1	28.1	28.1
26	22.6	22.6	22.6	22.6
27	22.8	22.8	22.8	22.9
others 1'	40.5	40.5	40.1	41.3
2'	25.5	25.5	25.4	25.2
3'	23.4	23.4	23.4	23.4
4'	14.1	14.1	14.1	14.1
acetate		170.3		
		21.8		

Table 10. ^{13}C NMR data

Carbon number	(184)[294,295]	(185)[294,295]	(174)	(177)[293]★
1	32.1	36.6	35.8	37.0
2	35.7	36.6	33.8	34.2
3	69.9	71.6	198.1	200.6
4	42.1	43.5	117.2	126.2
5	41.3	44.4	174.7	168.8
6	28.7	28.8	77.1	73.1
7	31.6	32.1	37.7	38.5
8	35.7	35.6	34.2	29.7
9	54.4	54.7	53.2	53.6
10	35.1	36.1	38.5	38.0
11	21.1	21.3	20.9	21.0
12	40.2	40.2	39.2	39.5
13	42.7	42.7	42.5	42.5
14	56.7	56.6	55.5	55.9
15	24.3	24.3	24.0	24.1
16	28.3	28.3	28.0	28.1
17	56.4	56.4	56.0	56.1
18	12.2	12.1	10.0	12.0
19	11.3	11.9	18.5	19.5
20	35.9	35.8	35.8	35.7
21	18.7	18.7	18.7	18.6
22	36.3	36.3	36.1	36.1
23	23.9	23.9	23.8	23.8
24	39.6	39.6	39.6	39.6
25	28.1	28.1	27.9	28.0
26	22.6	22.6	22.6	22.5
27	22.9	22.8	22.8	22.8
others 1'	34.2	36.6		

★ spectra recorded at 100.16 MHz

Table 11. ^{13}C NMR data

Carbon number	(167)	(166)	(171)	(169)	(164)
1	28.7*	29.6*	27.8	31.0	31.2
2	27.1	23.2	27.0	25.9	26.0*
3	66.0	68.0	63.3*	66.9*	64.3**
4	64.9	62.0	63.2*	66.0*	63.8**
5	67.5	66.8	69.9	66.9*	69.0
6	29.8	30.4	28.5	30.4	30.4
7	28.5*	29.8*	29.9	26.2	26.4*
8	35.7	35.5**	35.5	35.2	35.2
9	50.1	50.0	50.6	46.1	47.3
10	35.7	35.3**	35.5	36.2	36.2
11	21.0	21.1	20.8	21.3	21.4
12	39.9	39.8	39.8	39.9	39.9
13	42.7	42.7	42.7	42.6	42.7
14	56.4	56.3	56.3	56.2**	56.2***
15	24.2	24.2	24.2	24.3	24.3
16	28.2	28.2	28.2	28.2	28.4
17	56.4	56.3	56.3	56.4**	56.4***
18	12.1	21.1	12.0	12.0	12.0
19	17.8	17.5	17.3	18.7	19.0
20	35.8	35.8	35.8	35.8	35.8
21	18.7	18.7	18.7	18.7	18.7
22	36.3	36.2	36.2	36.2	36.2
23	23.9	23.9	23.9	23.9	23.9
24	39.6	39.6	39.6	39.6	39.6
25	28.1	28.1	28.1	28.1	28.1
26	22.6	22.6	22.6	22.6	22.6
27	22.8	22.8	22.8	22.9	22.8
acetate		170.2 21.5			

* chemical shifts may be interchanged

** chemical shifts may be interchanged

*** chemical shifts may be interchanged

Table 12. ^{13}C NMR data

Carbon number	(163)	(165)	(170)	(122)
1	35.5	35.1	31.8	39.6
2	29.6	25.2	27.9	37.7
3	68.0	71.0	64.4	210.2
4	123.4	119.0	120.7	48.3
5	147.8	149.7	150.5	138.6
6	33.2	33.0	32.5	122.9
7	32.3	32.4	32.9	31.9
8	36.2	36.0*	35.9	31.9
9	54.6	54.3	54.2	49.2
10	37.4	37.4	37.6	36.9
11	21.1	21.5	21.6	21.4
12	40.0	39.9	40.0	39.7
13	42.5	42.5	42.6	42.4
14	56.3	56.2	56.3	56.7
15	24.3	24.3	24.3	24.3
16	28.2	28.2	28.2	28.2
17	56.3	56.2	56.3	56.2
18	12.0	12.0	12.0	11.9
19	19.0	18.9	18.1	19.2
20	35.8	35.9*	35.9	35.8
21	18.7	18.7	18.7	18.7
22	36.3	36.3	36.2	36.2
23	23.9	23.9	23.9	23.9
24	39.6	39.6	39.6	39.6
25	28.1	28.1	28.1	28.1
26	22.6	22.6	22.6	22.6
27	22.9	22.8	22.8	22.8
acetate		171.0		
		21.1		

* chemical shifts may be interchanged

Table 13. ^{13}C NMR data

Carbon number	(172)[296]	(279)★	(152)[297]	(158)
1	33.9	35.3	39.2	36.6
2	23.1	21.2	33.5	29.5
3	129.2	26.6	60.2	74.5
4	124.9	123.2	43.5	40.0
5	141.6	134.0	141.0	139.7
6	123.2	126.8	122.5	123.0
7	31.9	129.3	31.9	31.9
8	31.9	37.9	31.9	31.9
9	48.6	40.1	50.2	50.1
10	35.2	38.4	36.4	37.2
11	21.1	20.9	21.1	21.1
12	39.9	39.9	39.8	39.8
13	42.5	42.4	42.4	42.4
14	57.1	56.1	56.8	56.8
15	24.2	24.1	24.3	24.3
16	28.3	28.2	28.2	28.2
17	56.3	56.2	56.3	56.3
18	12.0	12.0	11.9	11.9
19	18.8	16.3	19.3	19.3
20	35.8	35.8	35.8	35.8
21	18.8	18.6	18.8	18.8
22	36.3	36.1	36.3	36.3
23	23.9	23.9	23.9	23.9
24	39.6	39.5	39.6	39.6
25	28.1	28.2	28.1	28.1
26	22.6	22.6	22.6	22.6
27	22.8	22.8	22.8	22.8

★ spectra recorded at 100.16 MHz

Table 14. ^{13}C NMR data

Carbon number	(186)	(280)[298]	(187)	(189)★
1	28.6	27.7	29.3	23.8
2	30.6	30.5	20.6*	27.9
3	67.2	65.4	20.2*	67.4
4	45.0	43.5	35.9	43.5
5	73.9	82.5	72.0	75.8
6	133.3	131.0	133.9**	133.9
7	133.3	133.2	132.8	129.0
8	38.5	38.1	38.5	39.4*
9	40.1	38.9	40.1	37.3
10	38.3	38.3	40.4	38.9
11	21.1	20.7	20.7*	21.1
12	40.9	40.0	40.2	40.8
13	43.6	43.6	43.8	43.3
14	54.0	53.5	54.1	54.4
15	23.9	23.7	23.9	23.8
16	28.4	28.5	28.4	28.3
17	56.2	56.0	56.2	56.0
18	12.2	12.0	12.2	11.9
19	14.7	15.2	14.5	17.7
20	35.9	35.6	35.9	35.7
21	18.7	18.6	18.7	18.6
22	36.2	36.0	36.3	36.1
23	23.9	23.7	24.0	24.7
24	39.6	39.3	39.6	40.0*
25	28.1	28.2	28.1	27.9
26	22.6	22.5	22.6	22.5
27	22.8	22.8	22.8	22.8

★ spectra recorded at 100.16 MHz

* chemical shifts may be interchanged

** chemical shifts may be interchanged

Table 15. ^{13}C NMR data

Carbon number	(199)★	(203)★	(194)★	(156)
1	28.9	31.9	32.1	33.5
2	22.6	22.6	27.5	20.7*
3	130.7	130.7	63.9	20.5*
4	133.6	133.6	116.9	31.2
5	71.9	72.0		74.1
6	35.1	35.1	68.8	76.3
7	27.7	26.7	42.2	34.9
8	34.8	34.8	34.2	30.2
9	43.2	43.2	53.6	42.6
10	39.0	37.3	38.0	38.9
11	22.4	22.6	21.5	20.7*
12	40.0	39.9	39.4	40.1
13	42.5	42.5	42.2	42.8
14	56.3*	56.2	55.7*	56.1
15	24.2	24.2	24.2	24.2
16	28.2	28.2	28.0	28.2
17	56.2*	56.2	56.1*	56.4
18	11.9	11.9	11.9	12.2
19	16.4	14.1	19.0	16.7
20	35.7	35.7	35.7	35.8
21	18.6	18.6	18.6	18.7
22	36.1	36.1	36.0	36.2
23	23.8	23.8	23.8	23.9
24	39.5	39.5	39.6	39.6
25	28.0	28.0	28.0	28.0
26	22.6	22.6	22.6	22.6
27	22.8	22.8	22.8	22.8

★ spectra recorded at 100.16 MHz

* chemical shifts may be interchanged

** chemical shifts may be interchanged

Table 16. ^{13}C NMR data

Carbon number	(176)★		(132)	(278)	(133)★
1	32.8		30.2	28.2	28.0
2	35.1		32.4	29.6	29.0
3	73.5		67.6*	66.5	68.5
4	43.9*		34.3**	29.9	35.9
5	63.1		89.6	86.2	76.5
6	64.0		68.0*	68.5	75.4
7	32.6		34.5**	34.2	34.1
8	29.9		29.8	29.9	30.0
9	51.0		45.8	45.6	45.5
10	35.7		40.1	40.1*	39.3
11	21.9		22.3	20.9	20.9
12	40.1		39.6	40.0*	39.9
13	42.4		42.8	42.8	42.9
14	56.3		56.4	56.2	55.8*
15	24.2		24.2	23.9	24.1
16	28.2		28.1	28.2	28.3
17	56.3		56.4	56.2	56.2*
18	11.9		12.2	12.3	12.1
19	16.9		17.3	16.9	16.5
20	35.7		35.8	35.8	35.8
21	18.6		18.7	18.7	18.6
22	36.1		36.2	36.2	36.1
23	23.8		23.9	23.9	23.9
24	39.5		39.6	39.6	39.5
25	28.1		28.1	28.1	28.2
26	22.8		22.6	22.6	22.5
27	23.0		22.8	22.8	22.8
others 1'	14.1	acetate	21.2	21.4	
2'	23.4		170.4	171.8	
3'	25.4				
4'	43.4*				

* chemical shifts may be interchanged

** chemical shifts may be interchanged

★ spectra recorded at 100.16 MHz

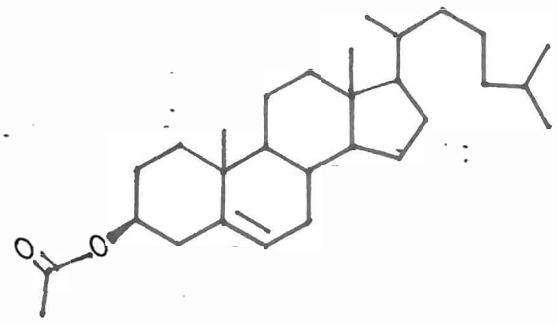
RESULTS AND DISCUSSION-I

RESULTS AND DISCUSSION-I

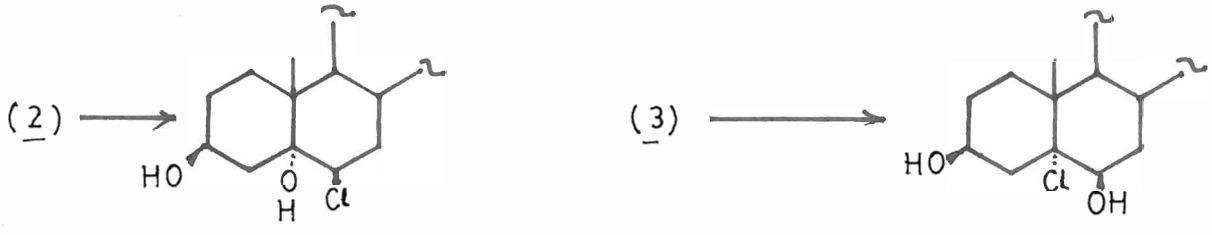
Preparation of 5,6-epoxysteroids

In a first investigation of the reaction of perbenzoic acid with cholesterol (1) and cholesterol acetate (116), Westphalen[125] isolated two products described as cholesterol "α" and "β" oxide. The first proved to be a pure compound and was later found to have the 5α,6α-configuration (2). The "β" oxide, subsequently prepared and studied by Ruzicka and Bosshard,[126] crystallized well and had reproducible properties, but was recognized by Hattori[127] in Japan (1940) as a 1:1 complex of the α- and the true β-epoxide (3). These two epoxides were distinguished on reaction with hydrogen chloride in chloroform[127,128] or with pyridine hydrochloride.[129] The cholesterol 5α,6α-epoxide (2) was cleaved with inversion at C6 and afforded 6β-chlorocholestane-3β,5α-diol (117), while the cholesterol β-epoxide (3) was cleaved by hydrogen chloride at the bond extending to C5 and afforded 5α-chlorocholestane-3β,6β-diol (118). Later on, these were also characterized by other workers.[130-132]

Cholesterol-α-epoxide (2) was prepared by epoxidation with m-chloroperbenzoic acid in almost quantitative yield according to Fieser's procedure.[82] Here, the electrophilic attack of peracid takes place from the less hindered α-face of the olefin giving rise of α-epoxide (Figure 43). The "molecular" mechanism of peracid epoxidation suggested by Bartlett[133,134] is more favoured than the 1,3-dipolar addition mechanism and is represented[135] in Figure 43.

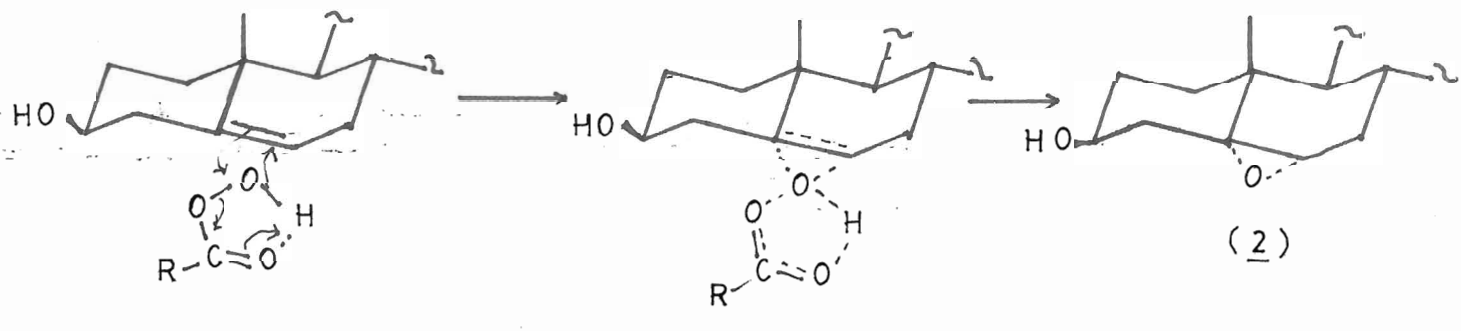


(116)

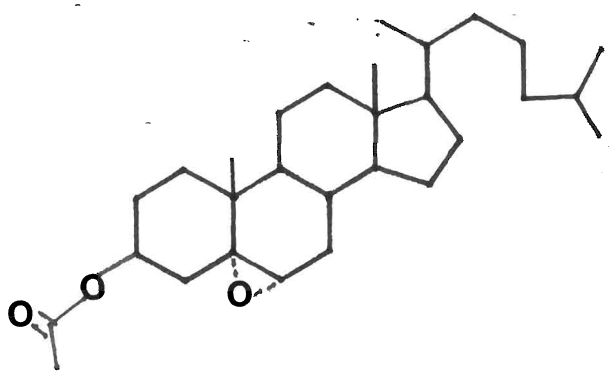


(117) (118)

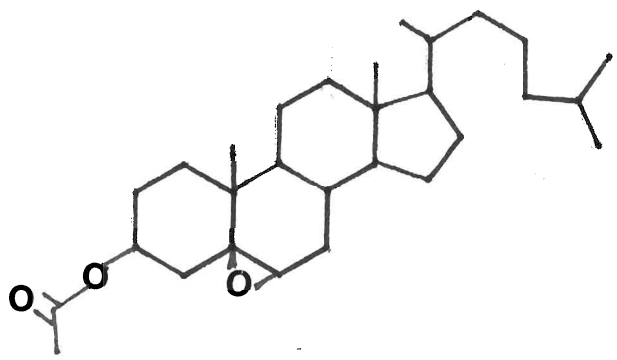
Figure 43



+ RCOOH



(2A)



(3A)

The cholesterol-β-epoxide (3) was prepared by the addition of acetyl hypobromite (AcOBr) across the double bond in cholesteryl acetate (116) followed by ring closure and hydrolysis of 5α-bromocholestane-3β,6β-diol-diacetate (119)[83] (Figure 44).

The addition of acetyl hypobromite (AcOBr) prepared[83] from silver acetate and bromine in CCl_4, gave a poor yield of bromo derivative (119). In the present study (119) was also prepared in one step using lithium acetate and N-bromoacetamide at room temperature in approximately 80% yield.[84] On refluxing (119) with 5% methanolic sodium hydroxide solution, pure 5,6β-epoxide (3) was obtained.

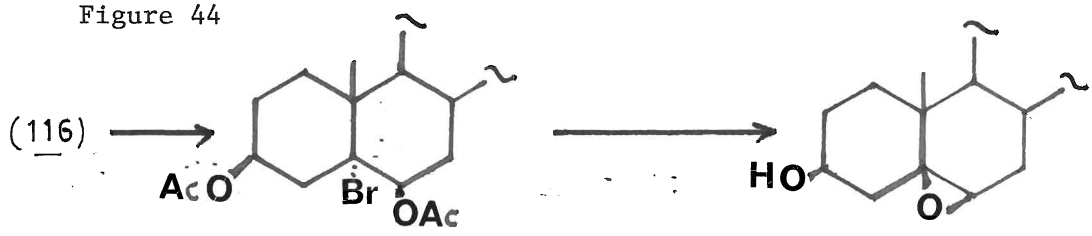
Masahiko et al.[136] described β-epoxidation of cholesterol in one step using ferric acetyl acetonate in acetonitrile in 68% yield. However, it was also accompanied by α-epoxide (17%), 5α-cholestane-3β,5,6β-triol (4) (5%), and a mixture of 7α- and 7β-hydroxycholesterol (120) (5%), and therefore this procedure was not used in the present study.

The preparation of 5,6α- and 5,6β-epoxides with α-stereochemistry of the 3-hydroxyl group, required the preparation of Δ^5-cholesten-3α-ol (epicholesterol (121)).

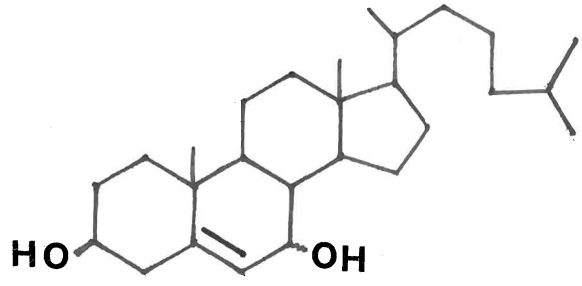
Corey et al.[137] have reported the preparation of epicholesterol in 56% yield from p-toluenesulfonate of cholesterol by inverting the configuration at C3 using KO_2 and 18-crown ether in DMSO-DME (1:1). However, this procedure was not used because of its irreproducibility.[138]

Ishige and Shiola[94] reported an excellent method for the preparation of epicholesterol (121) from Δ^5-cholesten-3-one (122). The hydrogenation

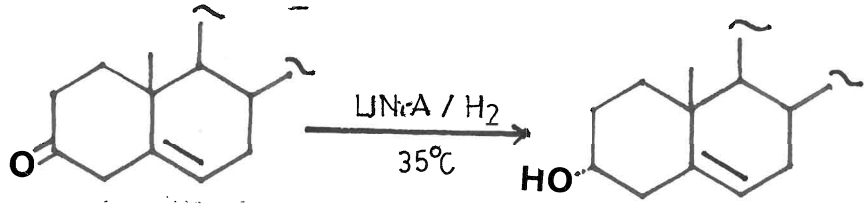
Figure 44



(119) (3)



(120)



(122) (121)

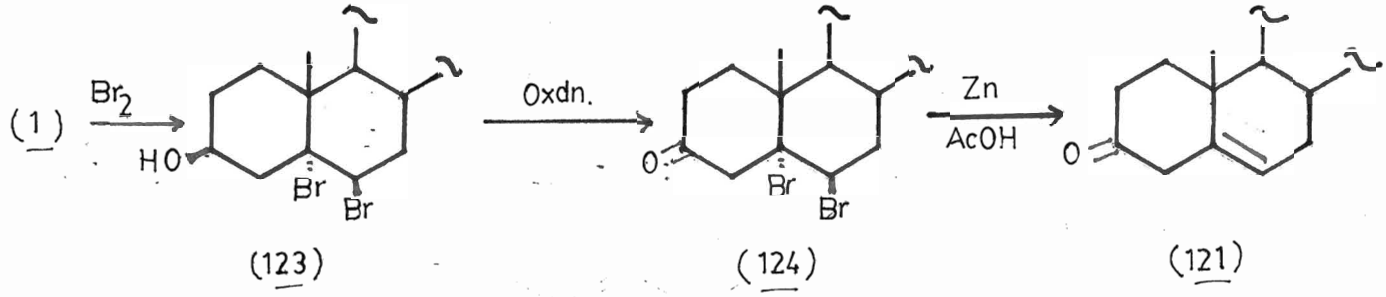
of (122) using Urushibara nickel A (UNiA) catalyst[95,140] in cyclohexane at 35°C for about 3 hours while stirring at 1000 cycles/minute at 100 kg/cm^2 pressure afforded mainly (>95%) epicholesterol (121).

The classical procedure[93] for the preparation of (122) was undertaken in the present study. Cholesterol (1) was first brominated to dibromide (123), followed by oxidation of 3-OH to 5α,6β-dibromocholestan-3-one (124), which on debromination with zinc dust gave pure Δ^5-cholesten-3-one (122) in approximately 70% yield from cholesterol.

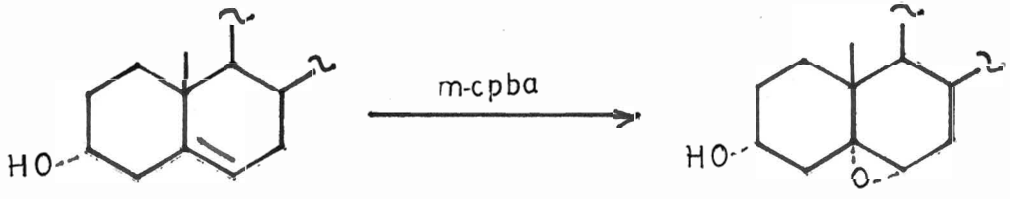
The epoxidation of epicholesterol (121) using m-chloroperbenzoic acid afforded only 5α,6α-epoxycholestan-3α-ol (125). The exclusive formation of α-epoxide can be interpreted in terms of hydrogen bonding between peracid and 3α-OH, which directs the peracid to epoxidize $\Delta^{5(6)}$ double bond from the α-face[97] (Fig. 45).

Epoxidation of 3α-acetoxycholest-5-ene

Due to the participation of 3α-OH group of epicholesterol, epoxidation occurs mainly from the α-face. Therefore, if the intermolecular hydrogen bonding between 3α-OH and the peracid is removed, the formation of β-epoxide would also be expected, along with α-epoxide. Canet and Guilleux[97] found that the use of ether (solvent) as competitor for hydrogen bonding with 3α-OH afforded only 5% of 5,6β-epoxycholestan-3α-ol (126) along with α-epoxide (125) (95%). The hydrogen bonding between 3α-OH and peracid can be removed by making its acetate (127).[97]

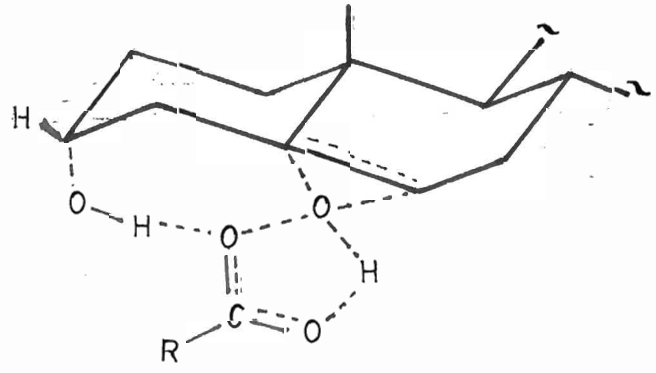


(123) (124) (121)



(121) (125)

Figure 45

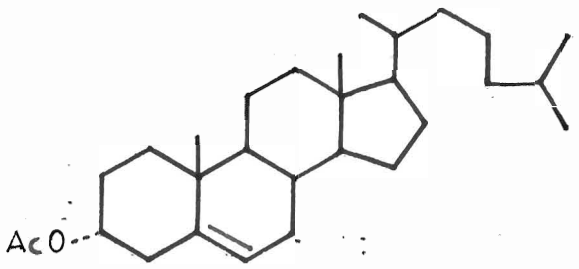


Epoxidation of epicholesteryl acetate (127) with mono perphthalic acid in ether has been reported[97] to give a triol monoacetate, m.p. 65° (128), which on reduction with $LiAlH_4$ gave cholestanetriol (133). On the other hand, epoxidation of (127) in anhydrous benzene gave a 67% mixture consisting of 53% 3α-acetoxycholestan-5α,6α-epoxide (129) and a gummy material, 3α-acetoxycholestane-5β,6β-epoxide (130) (47%) and 33% hydrolysis products.[97]

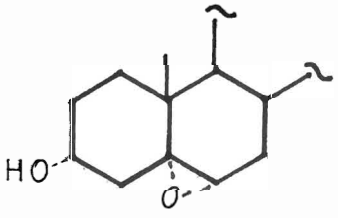
In the present investigation, the product distribution, in the epoxidation of epicholesteryl acetate (127) was somewhat more interesting.

Treatment of (127) with m-chloroperbenzoic acid in methylene chloride afforded a mixture. This mixture on fractional crystallization from acetone-hexane, gave a new compound, 3β,5β-oxidocholestan-6α-ol-6-acetate (131) in 25.8% yield.

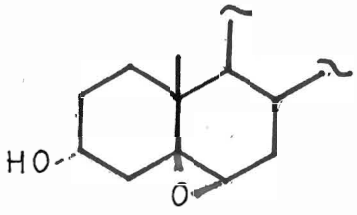
The mother liquor on evaporation indicated the presence of 3α-acetoxycholestan-5β,6β-epoxide (130) as the major component (indicated by ^1H NMR of the mixture). Since the acetate group was not required in the epoxide, for present purposes, the mother liquor was dissolved in methanol and hydrolyzed with 5% sodium bicarbonate solution (methanolic). Two compounds isolated after column chromatography were identified as 5β,6β-epoxycholestane-3α-ol (126) and unhydrolyzed 3α-acetoxy-5α,6α-epoxycholestane (125). The latter was further identified by comparing m.p., tlc and spectral data (^1H NMR, ^{13}C NMR and mass spec) with authentic sample prepared by normal acetylation on epicholesterol-α-epoxide (125).



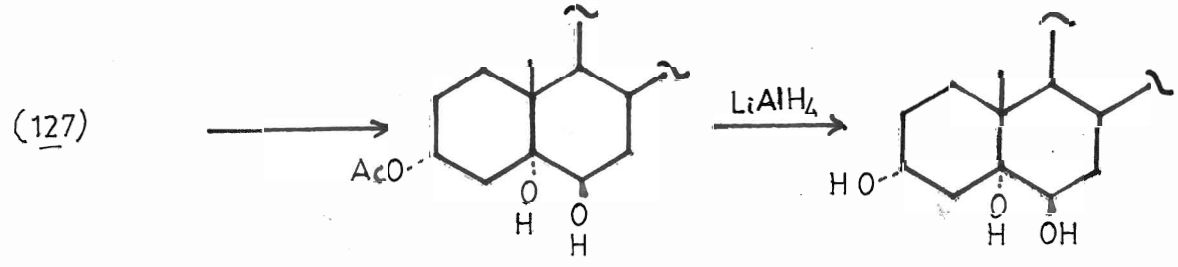
(127)



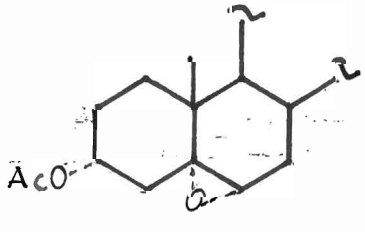
(125)



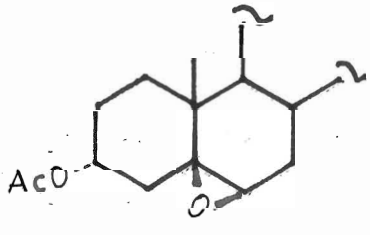
(126)



(128) (133)



(129)



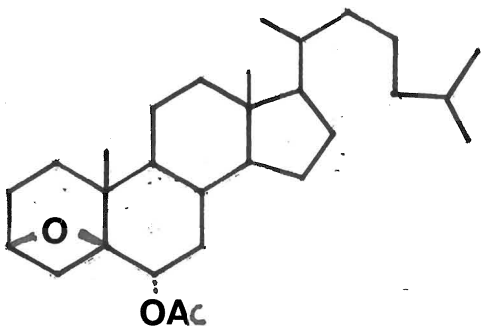
(130)

3β,5β-Oxidocholestan-6α-ol-6-acetate (131)

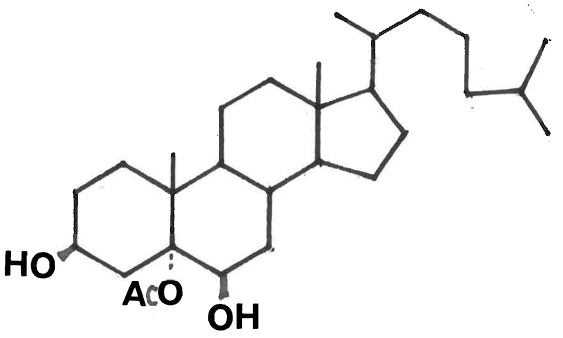
The compound obtained on fractional crystallization from the reaction mixture from the epoxidation of 3α-acetoxycholest-5-ene (127) was first thought to be 5α-cholestan-3β,5,6β-triol-5-acetate (132). This is because (131) had a melting point of 170-172°C in good agreement with that of (132) (lit. m.p.[109] 170°C), and the infrared spectrum of (131) exhibited an absorption band at 3400 and 1740 cm^{-1}. The tentative mechanism for the formation of (132) in this epoxidation may be represented in Figure 46. Model examination of (130) showed that the 3α-acetoxy group could attack through its carbonyl carbon at the C5 carbon of the epoxide, opening it in S_N2 manner.

Therefore, for comparison purposes, an authentic sample of 5α-cholestane-3β,5,6β-triol-5-acetate (132) was prepared from cholesterol-β-epoxide (3) by reacting with glacial acetic acid and sodium acetate. This authentic sample (132) showed significant differences in its ^1H NMR from that of (131). The ^1H NMR ($CDCl_3$) of (132) included signals at δ 3.15-3.70 (br. m., 1H, C-3Hα), 4.45-4.70 (m, 1H, C-6Hα), 1.98 (s, 3H, OAc) and 0.63 (s, 3H, C-18 methyl). While the ^1H NMR ($CDCl_3$) of (131) showed low field signals at different chemical shifts, viz. δ 4.65 (br. S) and 4.20 (br. S).

On several crystallizations from ethyl acetate-hexane, the m.p. of (131) was raised to 196-197°C. The structure of this compound was re-investigated and was tentatively assigned as an oxetane acetate, 3β,5β-oxidocholestan-6α-ol-6-acetate (131). The model studies revealed

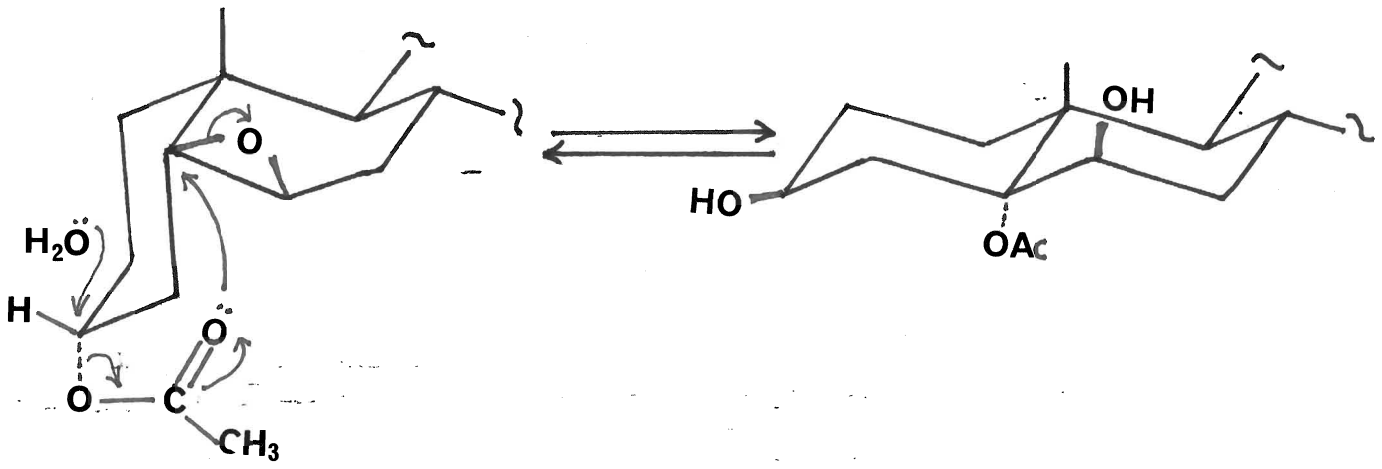


(131)



(132)

Figure 46



(130)

(132)

that 3α-acetoxy group of 3α-acetoxycholestane-β-epoxide (130) is in perfect location to attack the C6 carbon. The most probably mechanism for the formation of (131) will involve an S_N2 type attack of carbonyl oxygen of 3α-acetate group at C6 carbon from the α-face, and the opening of β-epoxide ring at C6, followed by its S_N2 type attack (intramolecular) at the C3 carbon from β side, displacing the 3α-acetoxy group completely to the 6α position (Figure 47). The later steps in the mechanism proposed may be concerted or sequential.

The absorption band at 3400 cm^{-1} in the infrared spectrum (KBr) of the oxetane-acetate (131) was most likely due to a molecule of water of crystallization which was reflected in its carbon, hydrogen analysis. The ^1H NMR spectrum was also consistent with oxetane structure (131).

The ^1H NMR ($CDCl_3$, 400 MHz) of (131) included signals at δ 4.65 (s, 1H, C6 Hβ), 4.20 (s, 1H, C3 Hα), 2.85 (α, 1H, $C4H_\alpha$, J_{gem} 16.0 Hz, low field wing of AB system), 2.15 (dxd, 1H, $C4H_\alpha$, J_{gem} 16.0 Hz, ν_{vic} 4.0 Hz, higher field wing of AB system), 1.13 (s, 3H, C19 methyl), 0.66 (s, 3H, C18 methyl) and 2.0 (s, 3H, OAc).

Irradiation of signal at δ 2.85 simplified the signal at δ 2.15 into a doublet indicating its geminal coupling with 4α-hydrogen. Irradiation of signal at δ 4.65 did not produce any change in the AB system, while irradiation of signal at δ 4.20 simplified the higher field wing of the AB system, and each doublet was collapsed into a singlet. This indicates the vicinal coupling of 4β-hydrogen with C-3α-hydrogen. Model studies showed that dihedral angle (ϕ) between 4α-H and 3α-H is nearly 80°, and therefore 4Hα is not coupling with 3Hα, which is in accordance with the

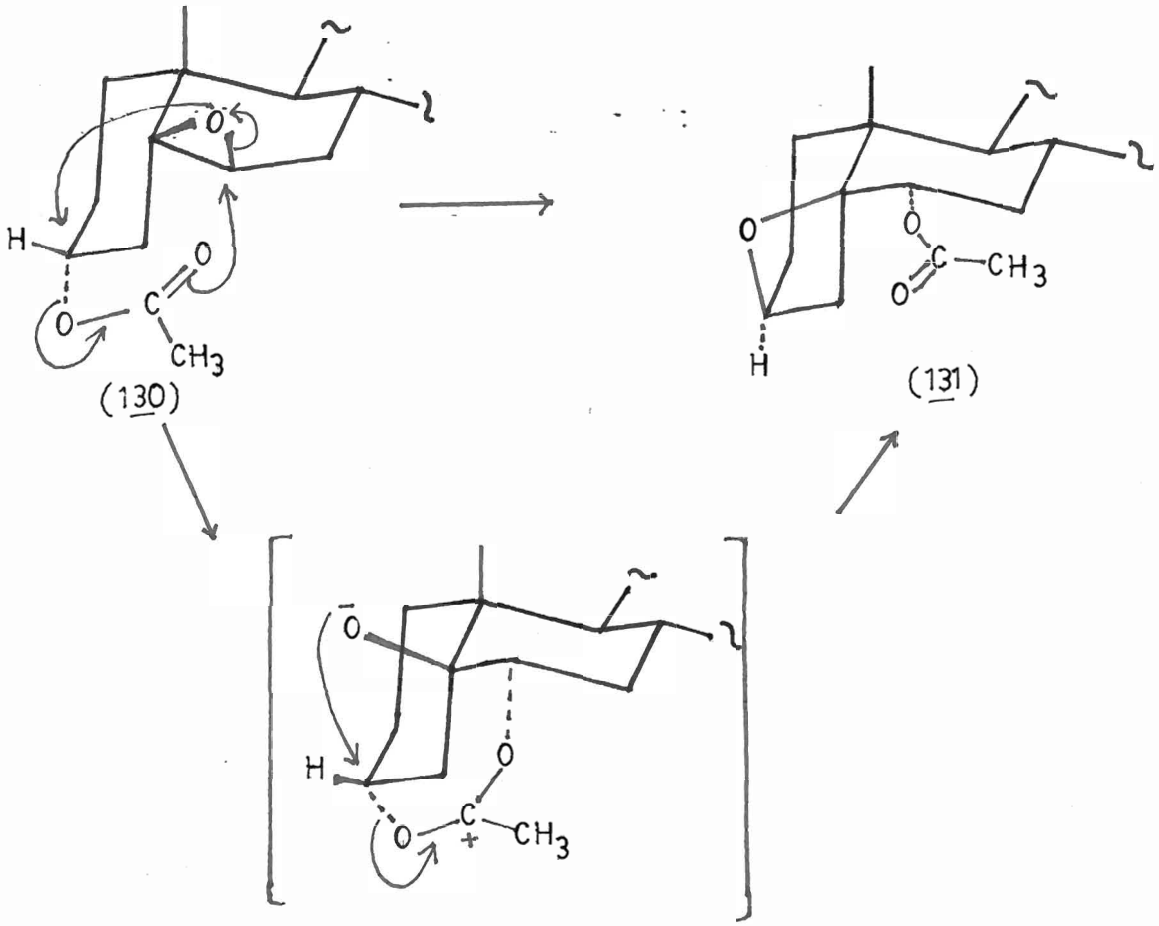
Karplus equation[141] for vicinal couplings. The 4β-hydrogen has a dihedral angle (ϕ) of approximately 30 degrees with 3Hα, which in fact split each signal of the doublet (higherfield wing of AB system) into doublet with ν_{vic} = 4.0 Hz. According to the Karplus equation, the coupling constant (ν_{vic}) for a dihedral angle of 30 degrees should be about 6.0 Hz, which is very close to the observed value.

The ^{1}H NMR can rule out the possibility of 5α-cholestane-3α,5,6β-triol-3-acetate (128), which was the only reported[97] product obtained on epoxidation of (127). They differ significantly in their melting points, m.p. 196-197° (131) and m.p. 65°C (128). The oxetane-acetate (131) on hydrolysis with lithium aluminum hydride afforded a compound which had the same R_f value as the starting material (131). Cholestan-3α,5α,6β-triol (133) because of carrying three hydroxyl groups is expected to have lower R_f value comparative to the hydrolyzed oxetane (134) in the same solvent.

The hydrolysis product of the oxetane acetate (131) failed to crystallize from any solvent system. Only gelatinous material was obtained, which on drying had an m.p. 160-163°C. On the other hand, the $LiAlH_4$ reduction product (133) of cholestane-3α,5α,6β-triol-3-monoacetate (128) has been reported[97] as crystalline material of m.p. 205-206°C. These experiments further supported the oxetane structure (131).

The structure of $LiAlH_4$ reduction product of (131) was tentatively assigned as 3β,5β-oxidocholestane-6α-ol (134). The ^{1}H NMR ($CDCl_3$) of oxetane (134) included the signals at 4.10-4.44 (m, 1H, C4 Hα), 3.50-3.70 (m, 1H, C6 Hβ), 3.10 (br s, 1H, C6 OH, exchangable with D_2O), 1.12 (s, 3H, C19 methyl), and 0.68 (s, 3H, C18 methyl). As expected, the signal at

Figure 47



δ 4.65 for C6 Hβ in (131) shifted upfield to δ 3.50-3.70 (m), and the lowfield wing of the AB system for C4 methylene hydrogens also moved to the upfield region and disappeared in the methylene signals of the steroid framework.

This hydrolysis experiment also removed the possibility of the structure 5α-cholestan-3β,5,6β-triol-5-acetate (132), as it, on hydrolysis, gives a well known 5α-cholestan-3β,5α,6β-triol (4). An authentic sample of (4) was prepared for spectral comparison.

The oxetane (134) exhibited significant differences in its spectral (^{1}H NMR, ^{13}C NMR, mass spec), tlc and m.p. from the authentic triol (4). These data further supported the oxetane structure (131).

The 3α,5α-oxidocholestan-6β-ol (135)[142] and its corresponding acetate derivative (136) have already been well characterized by Tsui et al.[98] but have no correlation with their β-isomers (134) and (131) respectively.

Supportive evidence for oxetane structure (131) also came from its rearrangement into 3α-acetoxy-5β,6β-epoxycholestane (130). The oxetane acetate (131) was completely converted into β-epoxide (130) when a dry sample was heated at 200°C for 30 minutes at 0.1 mm Hg. The probable mechanism for this rearrangement is represented in Figure 48. On standing at room temperature for 20 days in chloroform, the β-epoxyacetate (130) obtained from the thermal rearrangement, rearranged back into the oxetane-acetate (131).

In an attempt to study this interconversion, an authentic sample of 3α-acetoxy-5β,6β-epoxycholestane (130) was prepared by normal acetylation of 5β,6β-epoxycholestan-3α-ol (126). Since it was obtained as a gum,[97]

an effort to crystallize using several solvent systems made it more impure and showed an appreciable amount of a new compound which on tlc corresponds to oxetane acetate (131). The only isolated crystals from this were those of oxetane acetate (131), identified with mixed m.p., mixed tlc and ^{1}H NMR.

These interconversions of oxetane (131) and isomeric β-epoxide (130) provided further evidence supporting the oxetane acetate structure (131) (Figure 48). Model studies were in agreement with these rearrangements because of the stereochemistry at C3, C5 and C6 carbons which bring the acetoxy group in a position of easy transfer between C3 and C6 by an S_N2 type mechanism (Figs. 47 and 48). These rearrangements also fulfilled the requirements of inversion of configuration in the S_N2 mechanism at the carbons involved, which is one of the basic tenets of organic reaction mechanisms.[143]

Tsui and Just[98] have reported an analogous rearrangement of α-oxetane (135) into β-epoxide (126). 3α,5α-Oxidocholestan-3β-ol (135) obtained on reacting 3β-mesyloxycholestan-5α,6β-diol-6-acetate (137) with potassium t-butoxide along with β-epoxide (126), rearranged[98] to the latter (126) by submitting it to the reaction conditions (Fig. 49).

This[98] provided a literature precedent for the existence of oxetane systems and its conversion to the corresponding epoxide.

The possibility of 5α-cholestane-3β,5α,6β-triol-5-acetate (132) as a structure was further discarded since it has a trans A/B ring junction. It is highly unlikely for (132) to rearrange into oxetane acetate (131) containing comparatively strained and unstable cis A/B ring function.

Figure 48

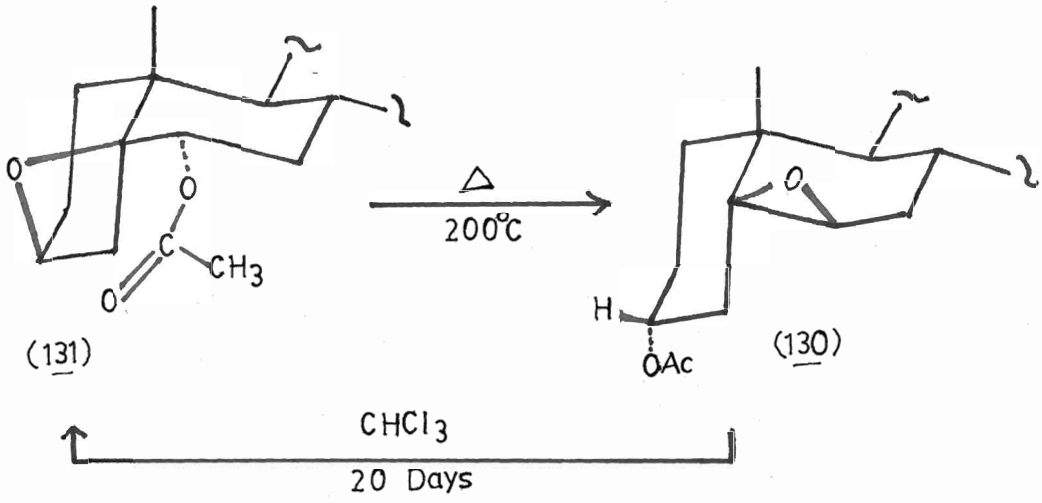
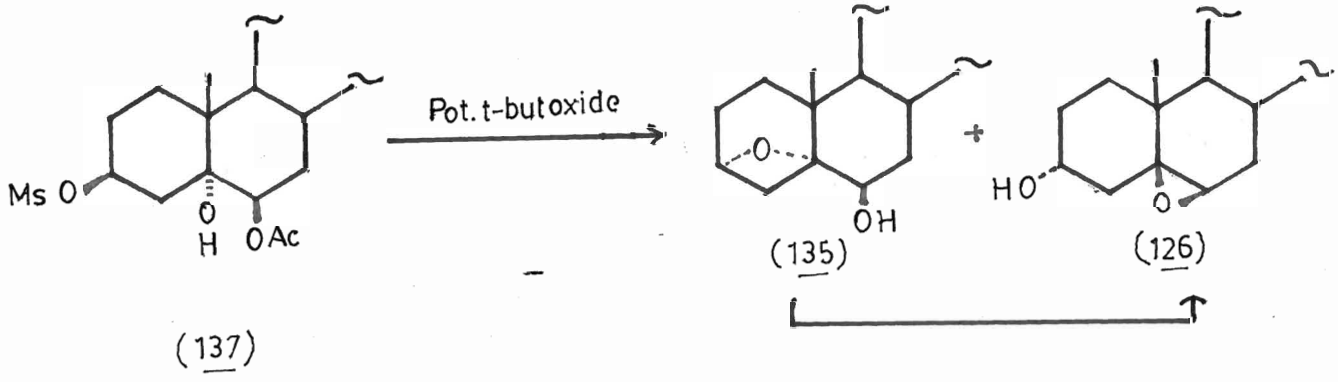
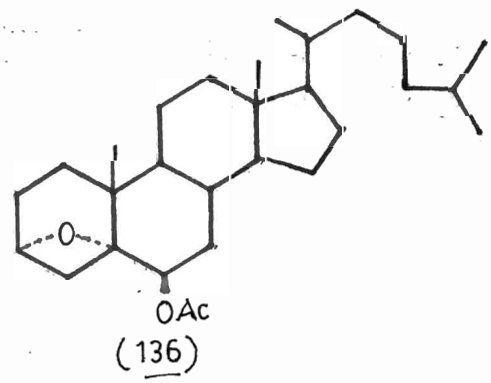


Figure 49



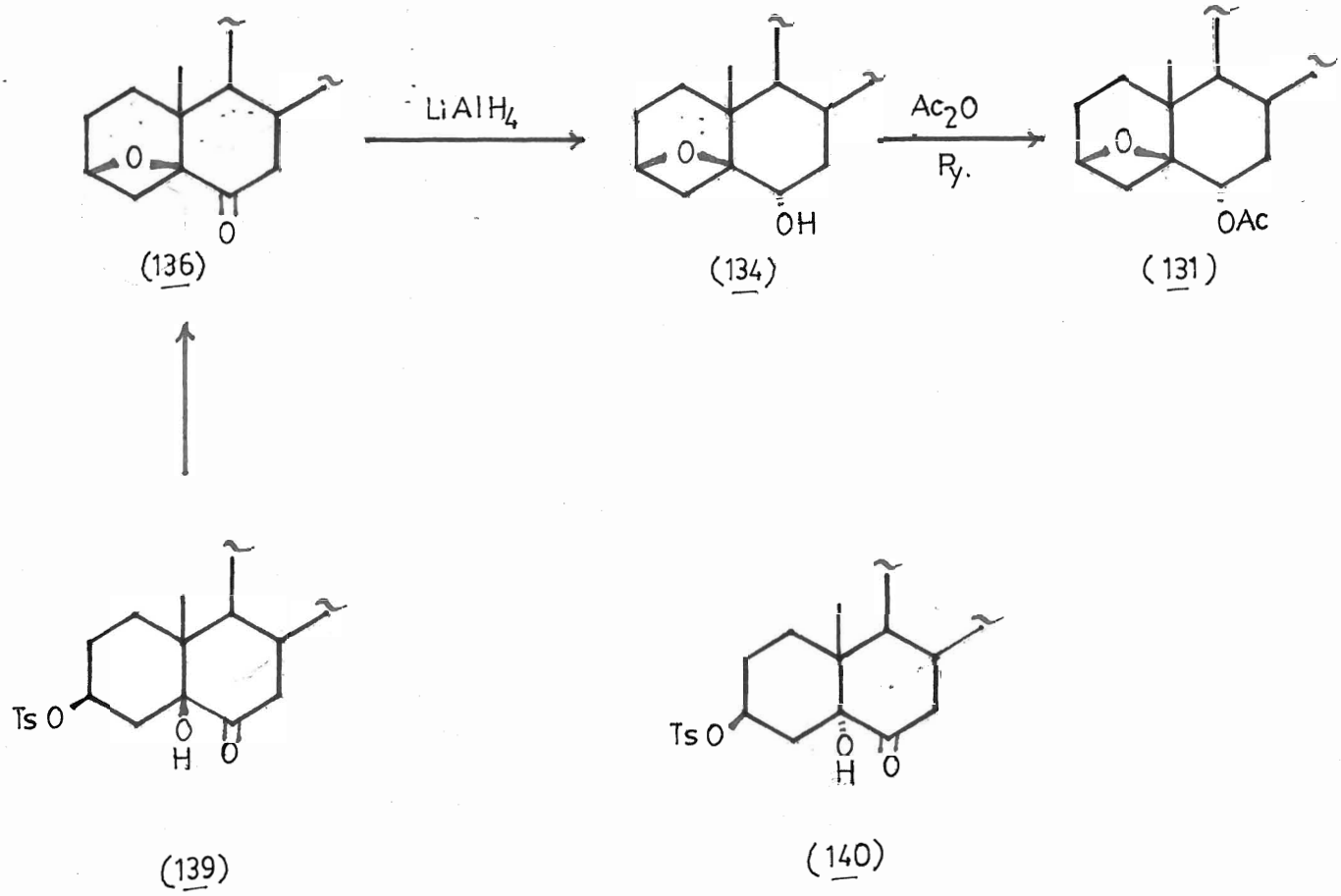


The oxetane acetate (132) has an historical significance. Its existence was reported as early as in 1966. A. T. Rowland[144] reported its preparation from 3β,5β-oxidocholestane-6-one (138) by reducing to corresponding alcohol (134) followed by acetylation (Fig. 50).

In this regard, the report[144-146] on solvolysis of 3β-tosyloxy-5β-hydroxycholestan-6-one (139) was of more than passing interest. The product obtained on hydrolysis using a variety of solvents was assigned the oxetanone structure (138), and it was suggested that the reaction proceeded via a transition state involving "partial bonding" from the oxygen at C5 to C3 before significant carbonium ion character at C3 is developed[145] (Fig. 51). Although the reaction caused "raised eyebrows"[147-148] it was not challenged until 1978.[149-150] Dave and Warnhoff's attention was attracted towards this unprecedented retention of configuration during an S_N2 substitution.[145] Moreover the C3 epimer[145] (140) with correct stereochemistry for a normal S_N2 reaction failed to produce the oxetanone (138) under similar conditions.[145]

Dave and Warnhoff[151] re-investigated the solvolysis of (139) and characterized the solvolysis product as 3α,5α-oxido-A-homo-B-norcholestan-4a-one (141) readily distinguishable by ^1H NMR spectroscopy in conjunction with deuterium exchange experiments from the structure (138) (Fig. 52). They proposed an ingenious mechanism for the formation of (141) (Fig. 53), which would not violate the S_N2 inversion rule by involving the participation of the C6 carbonyl oxygen of (139) in a displacement at C3 with concomitant stereochemically favourable trans A/B ring stereochemistry. Moreover, the mechanism (Fig. 53) could also account for the failure of

Figure 50



(136) (134) (131)

(139) (140)

Figure 51

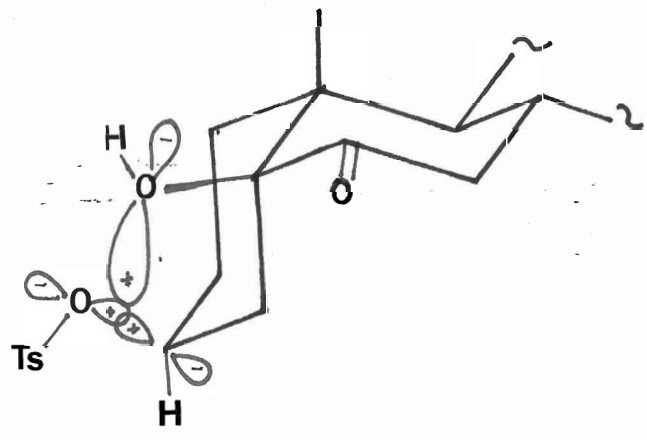
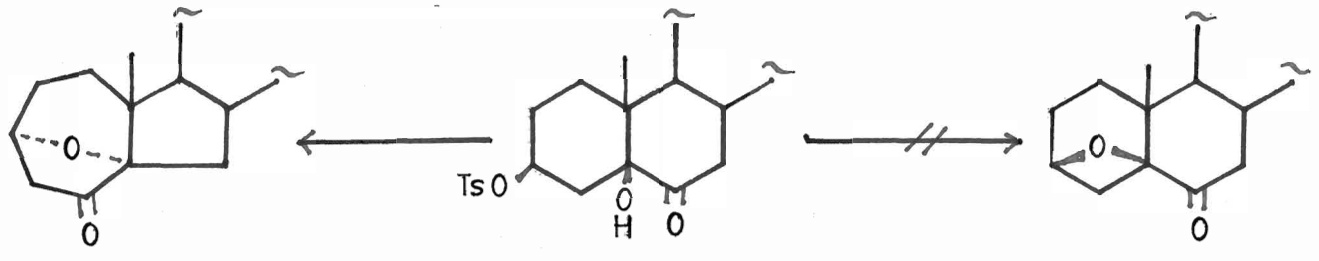
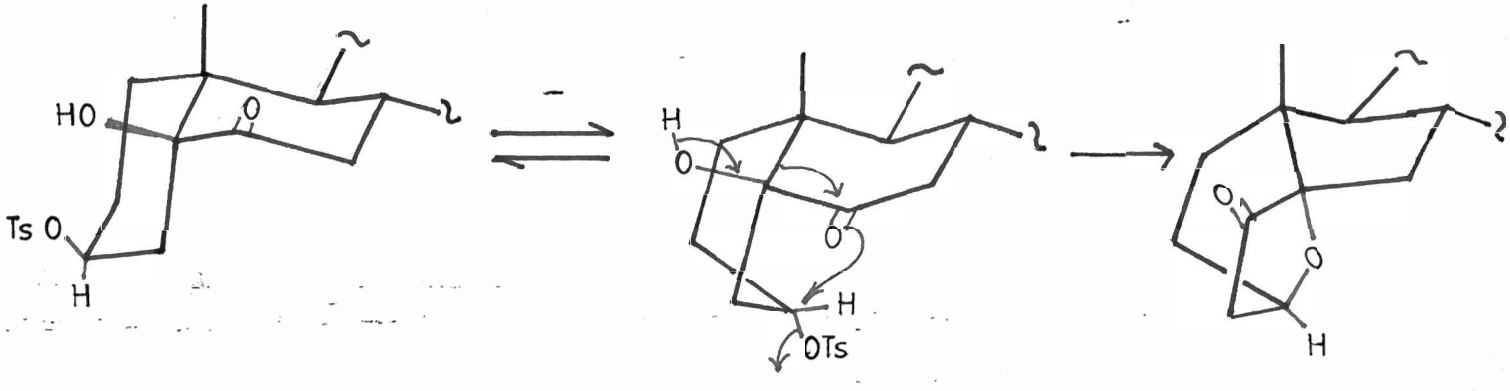


Figure 52



(141) (139) (138)

Figure 53



(139) (141)

C3 epimer (140) to do so with inversion.

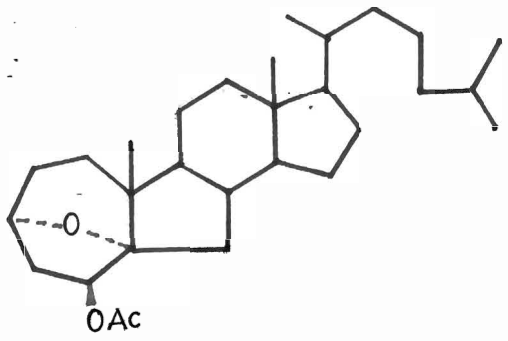
The compound prepared by Rowland[144,145] by $LiAlH_4$ reduction of (138) followed by its acetylation once thought to be the oxetane acetate (131), is, in fact, 3α,5α-oxido-A-homo-B-norcholestan-4aβ-ol-acetate (142).[153] This structure (142) may account for its unreactivity, as observed,[144] towards a number of acidic reagents. The oxetane (143), on the other hand, has been reported[152] to react with BF_3-etherate yielding cholesterol (Fig. 54). Richard and Marples[153] in 1979 reported the BF_3-catalyzed rearrangement of 3α,5α-oxidocholestan-6β-ol-6-acetate (136) yielding three products, 3α,5β-diol (145), 3α,10α-epoxide (146) and the 2α,5α-epoxide (147) (Fig. 55), but reported the failure with oxetane acetate (131) prepared according to Rowland's procedure. The α-oxetane (144) has also been shown to give epicholesterol[154] (Fig. 54).

In order to characterize oxetane acetate (131) with confidence, further investigations such as its behaviour towards acidic reagents are required. However, solving the structure (131) with x-ray diffraction is in progress.[155]

Other 5,6-epoxides

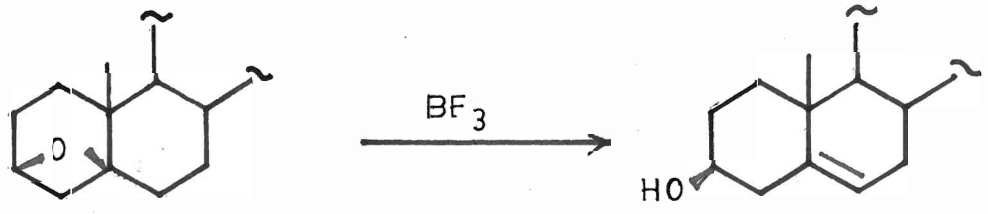
5α,6α-epoxycholestan-3,3-ethylenedioxyketal (150)[93] and 5β,6β-epoxycholestan-3,3-ethylenedioxyketal (151)[92] were prepared by epoxidation of 3,3-ethylenedioxycholestan-5-ene (149) according to the scheme shown in Figure 56.

5α,6α-Epoxycholestane (154)[19] was prepared by normal epoxidation of Δ^5-cholestene (153) with m-chloroperbenzoic acid in benzene. Δ^5-Cholestene

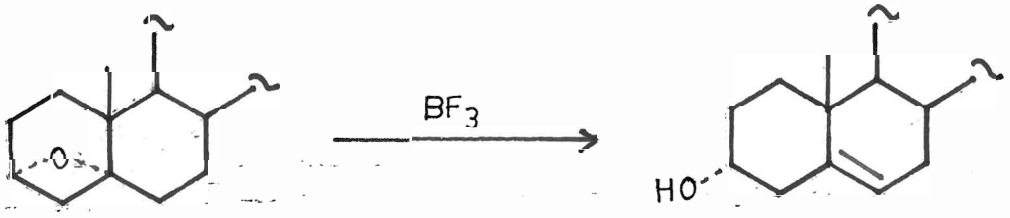


(142)

Figure 54

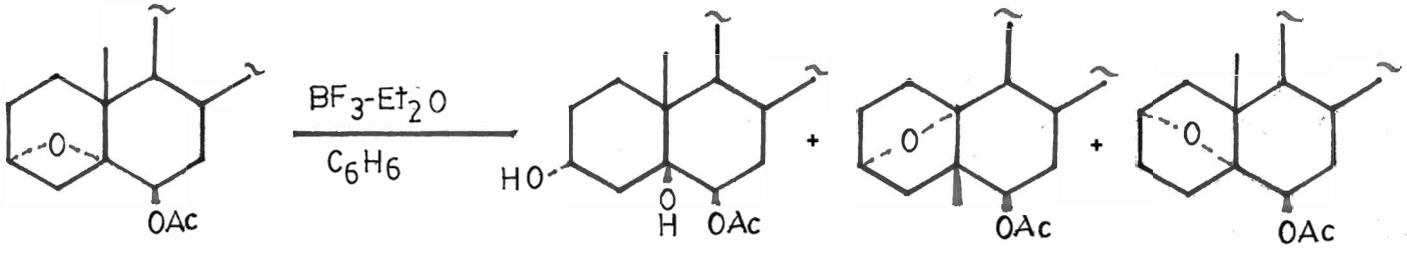


(143) (1)



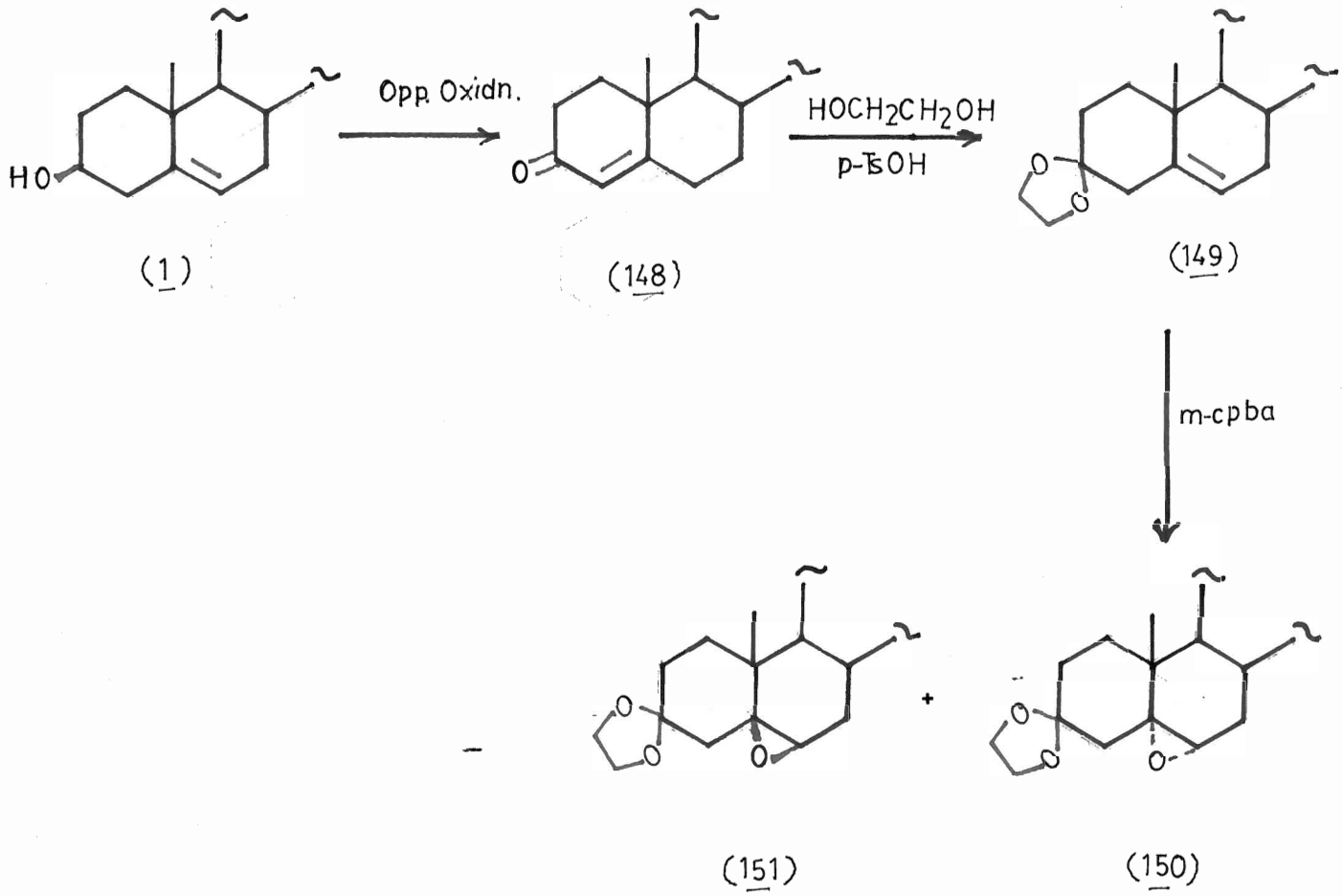
(144) (121)

Figure 55



(136) (145) (146) (147)

Figure 56



(1) (148) (149)

(151) (150)

(153) was obtained by reductive removal of chloride from cholesteryl chloride (152) following the procedure described by Turner et al.[86] (Fig. 57).

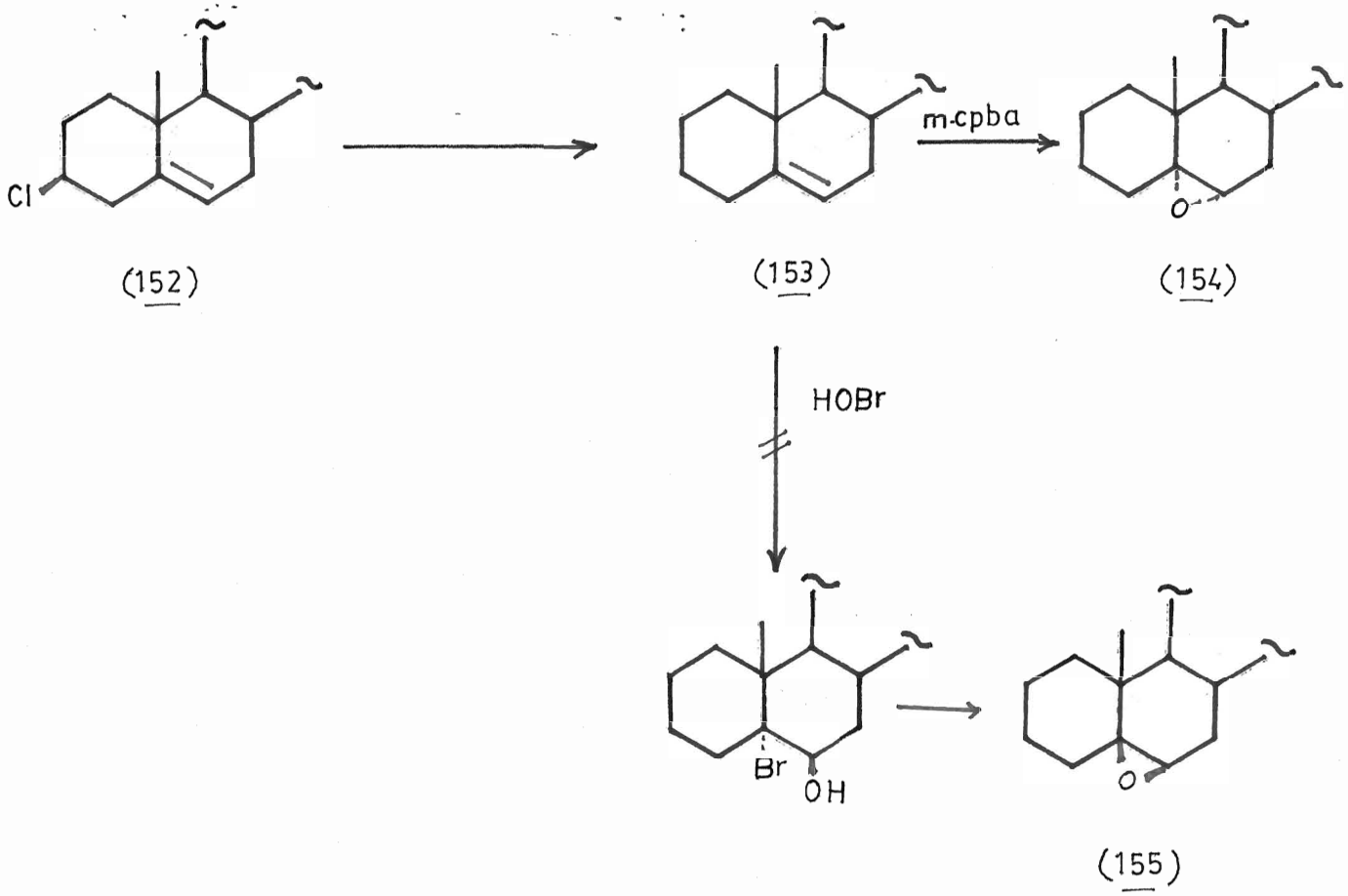
An obvious route to prepare 5β,6β-epoxycholestane (155) seemed to be the addition of hypobromous acid (HOBr) to provide the corresponding bromohydrin, and then its conversion to β-epoxide (155) (Fig. 57). Since the addition of hypobromous acid to (153) has been reported by Pavel Kocovsky[156] in 1980 to be a poor reaction, another procedure, that of Watabe and coworkers[15] (1981), was utilized in the present study. Three compounds, 5β,6β-epoxycholestane (155), cholestane-5α,6β-diol (156) and 5α-cholestan-5-ol-6-one (157) were obtained from this procedure (Fig. 58). This was in contradiction to the previous report[15] claiming the formation of only β-epoxide (155). However, N-bromosuccinamide (NBS) has been reported[88] to oxidize the 5α-cholestane-5,6β-diol (156) to 5α-hydroxy-cholest-6-one (157).

Epicholesteryl chloride (3α-chlorocholest-5-ene)

Shoppee[157] after a review of the literature concluded that owing to homoallylic participation[157,158] by the 5,6π bond, the hydroxyl group of cholesterol invariably undergoes replacement by chlorine with retention of configuration.

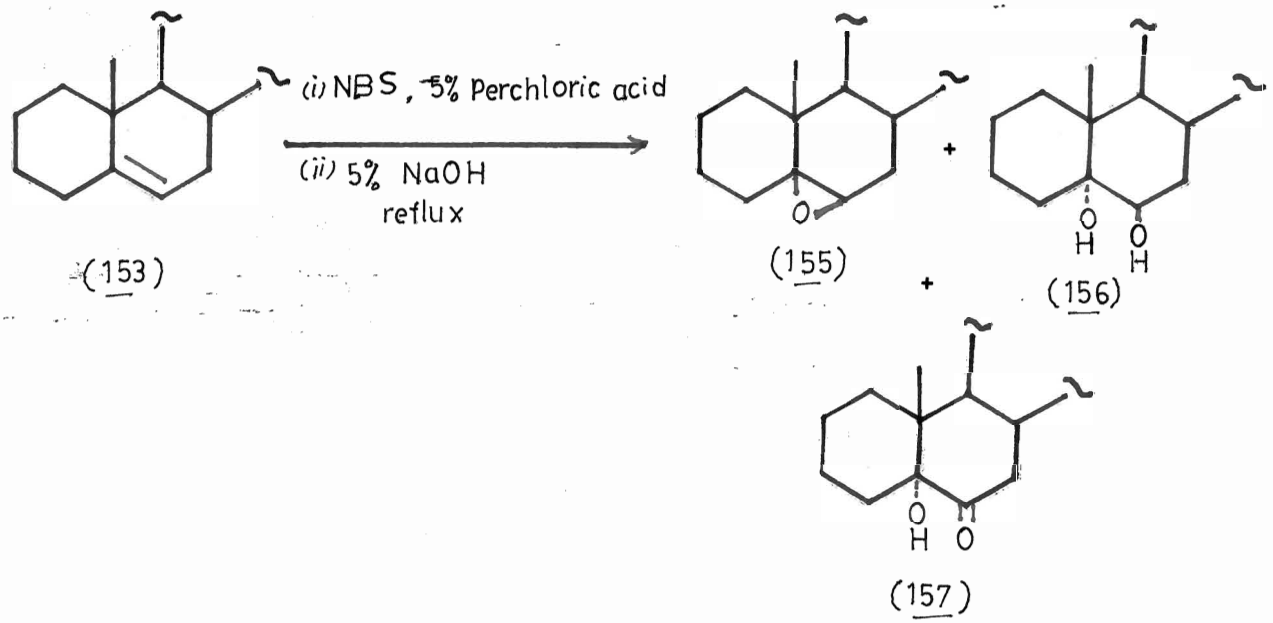
A usual route for cholesteryl chloride (152) preparation[159] is the reaction of cholesterol (1) with thionyl chloride. The retention of configuration at C3 occurs via S_Ni mechanism (Fig. 59), as thionyl chloride is well known for affecting S_Ni substitutions.[160]

Figure 57



(152) (153) (154) (155)

Figure 58



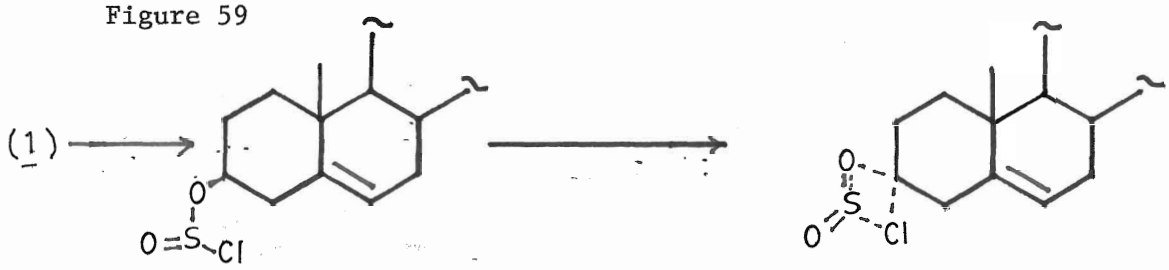
(153) (155) (156) (157)

In the present investigation, an attempt to prepare cholesteryl chloride (152) surprisingly resulted in the isolation of only epicholesteryl chloride (158) in more than 51% yield. As expected, it was less polar compared to (152) on tlc. The ^{1}H NMR revealed 3β-hydrogen of (158) shifted to lowfield at δ 4.50, compared to 3α-hydrogen in (152) which appears at δ 3.73,[113] and further confirmation of the epicholesteryl chloride structure (158) came from ^{13}C NMR spectrum.

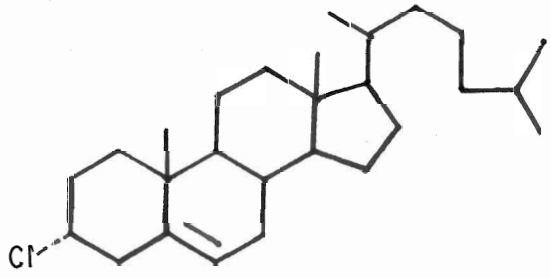
The above reaction was carried out in pyridine and in the excess of thionyl chloride. Since dipolar aprotic solvents are excellent media[161] for S_N2 reactions, it may be possible that instead of normal S_Ni[160] type substitution, the S_N2 mechanism was more favourable under the reaction conditions. Furthermore, the S_N2 type substitution might be aided by the excess of thionyl chloride, as inversion of configuration has been reported by Shoppee *et al.*[162] using the excess thionyl chloride during the transformation of a 2β-hydroxyl group to the 2α-chloro derivative (Fig. 60).

This may be a superior method over several other available methods either involving replacement;[116,163] with inversion of the 3β-hydroxyl group by chlorine prior to introduction of the 5,6π-bond by dehydration of a 5- or 6-ol, or using other reagents like triphenyl phosphine or triphenyl phosphite.[113,164] All these available methods either involve multi-steps, or give very low yields due to other side reactions, and therefore reflect the handicaps of these methods.

Figure 59

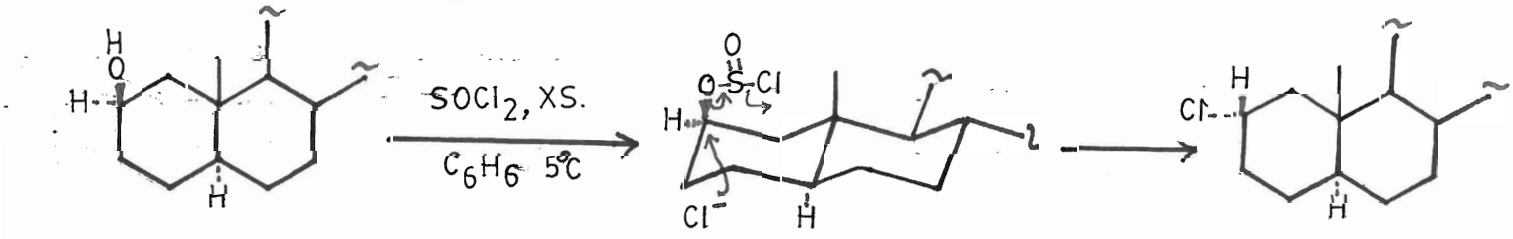


(152)



(158)

Figure 60



Preparation of 4,5-epoxides

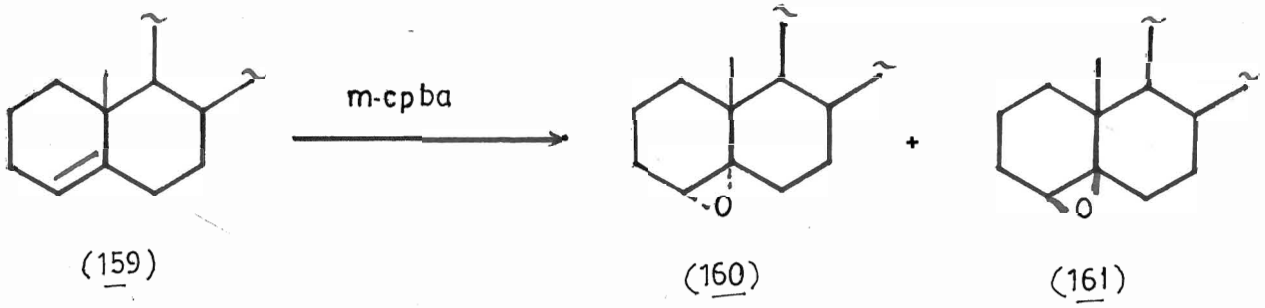
4α,5α-Epoxycholestane (160) and 4β,5β-epoxycholestane (161) were readily prepared by peracid epoxidation[41] of Δ^4-cholestene (159). The desirable Δ^4-cholestene (159) was obtained by refluxing the semicarbazone (162) of cholestenone with potassium t-butoxide (Fig. 61).

The synthesis of 4β,5β-epoxycholestan-3β-ol (164) was achieved by first reducing cholestenone (148) with sodium borohydride to 3β-hydroxy-cholest-4-ene (163),[103] then epoxidizing with m-chloroperbenzoic acid (Fig. 62). The epoxidation, here, occurs from the β-face because of the directing effect of the 3β-hydroxyl group by hydrogen bonding with peracid.[106]

In the preparation of 4α,5α-epoxycholestan-3β-ol (167);[106] the directing effect of 3β-hydroxyl group was eliminated by acetylating it (165). Peracid then epoxidizes the 4,5-double bond from the less hindered α-face giving 4α,5α-epoxycholestan-3β-ol-3-acetate (166) which on mild hydrolysis with potassium carbonate afforded the desired 4α,5α-epoxycholestan-3β-ol (167) (Fig. 62).

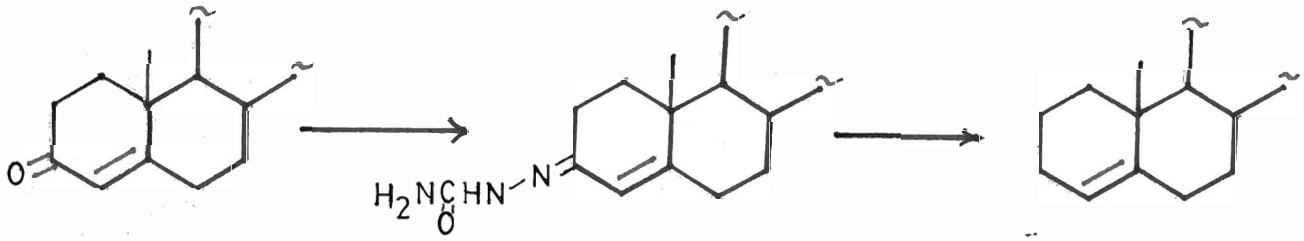
4β,5β-Epxoycholestan-3-one (168) was selectively formed on epoxidation of cholestenone (148) with alkaline hydrogen peroxide.[103,108] The epoxy ketone (168) on reduction with sodium borohydride[109] gave the desired 4β,5β-epoxycholestan-3α-ol (169) (Fig. 63).

In an attempt to prepare Δ^4-cholesten-3α-ol (170), the reduction of cholestenone (148) with diisobutyl aluminum hydride[165] could not result in its formation in more than 10% yield, and only Δ^4-cholesten-3β-ol (163)



(159) (160) (161)

Figure 61



(148) (162) (159)

Figure 62

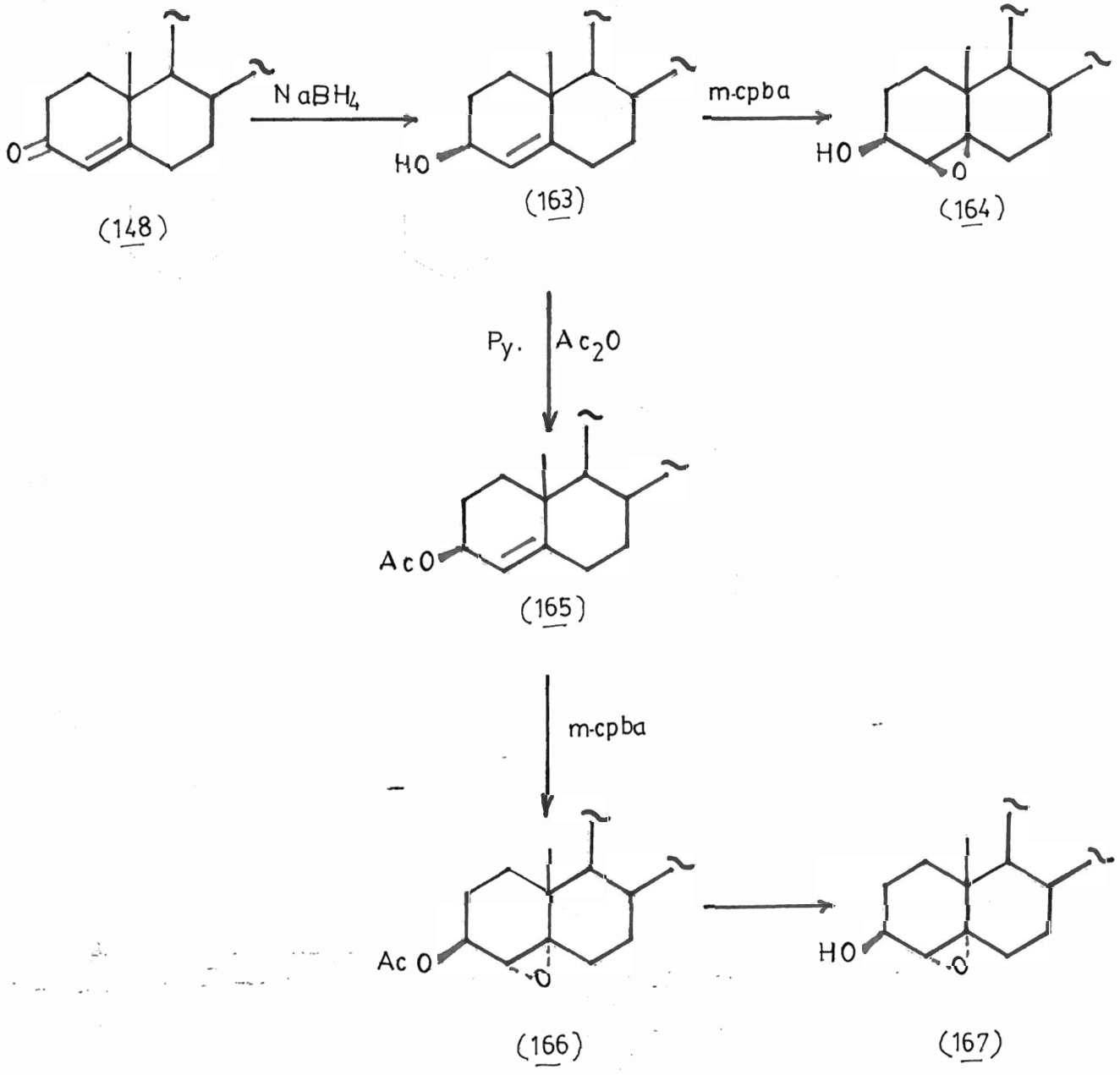
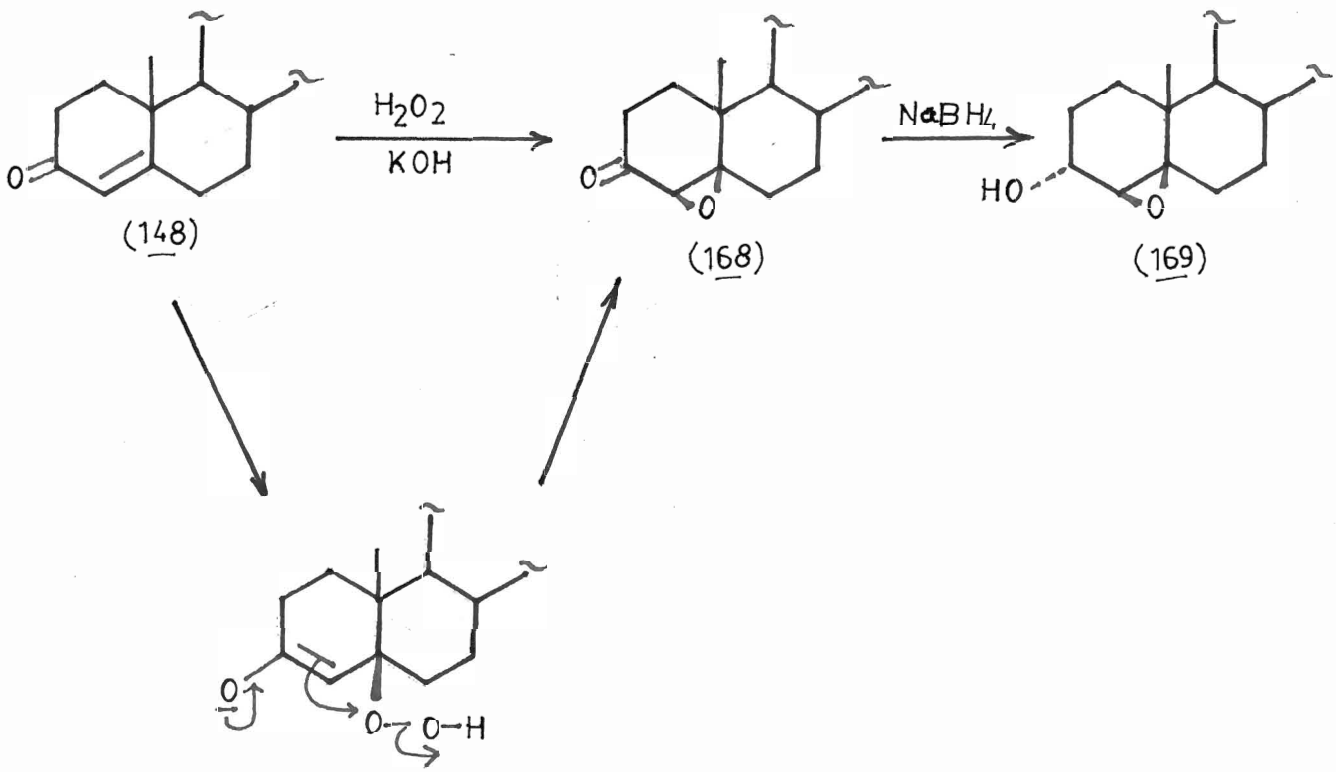
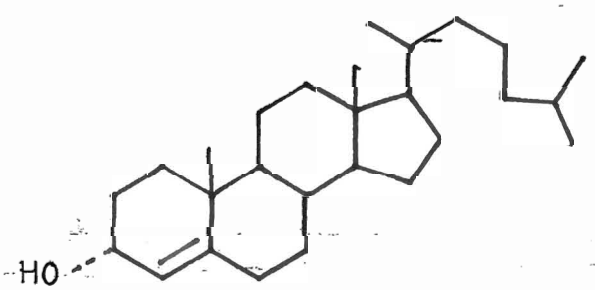


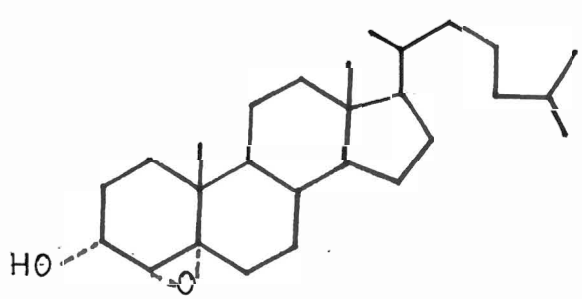
Figure 63



(148) (168) (169)



(170)



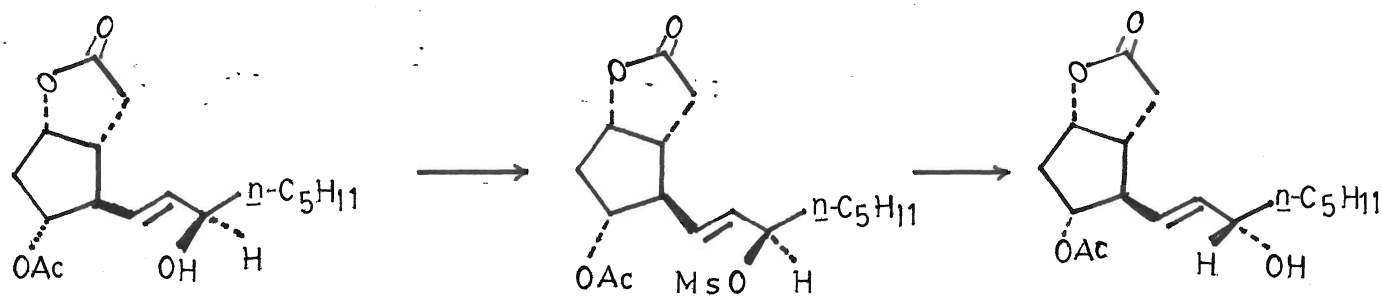
(171)

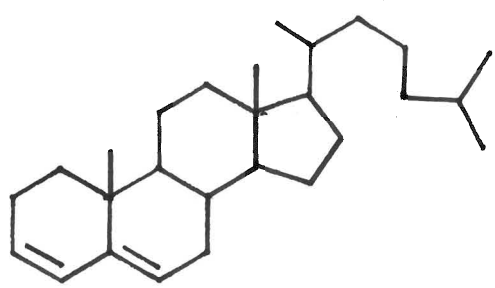
was isolated. Since (170) was desirable for the preparation of 4α,5α-epoxycholestan-3α-ol (171), a procedure involving the inversion of 3β-hydryoxyl to 3α-hydroxyl was attempted.

Corey et al.[137] reported the inversion of a β-allylic alcohol to the corresponding α-allylic alcohol during a prostaglandin synthesis. The procedure[137] employed involve first conversion of β-allylic alcohol to its methanesulfonate derivative, and the inversion of configuration was then achieved by nucleophilic attack of KO_2 (Fig. 64).

An attempt to mesylate Δ^4-cholesten-3β-ol (163) resulted in the formation of mainly $\Delta^{3,5}$-cholestadiene (172) even at -10°C, and no mesylate derivative was isolated. Addition of a few drops of water after 10 minutes of reaction at -10°C, showed the formation of mainly 3β-allylic alcohol (163) and its α-eipmer (170) as minor product. However, repeated attempts, under different conditions, could not reproduce the formation of appreciable amounts of α-allylic alcohol (170). And therefore the procedure used for inverting configuration in prostaglandins was found not useful to our allylic alcohol. Further experiments towards the preparation of (170) and therefore (171) are in progress.

Figure 64





(172)

EPOXIDE REACTIONS WITH STRONG ORGANIC BASES

In an epoxide molecule, the carbon and oxygen atoms of the oxirane ring provide electrophilic and nucleophilic centres as indicated in Figure 65. In addition to the carbon atoms of the oxirane, the β-hydrogen atoms can also act as electron acceptors. Various strong organic bases can bring about rearrangements of epoxides by abstracting α, β and higher hydrogens. The results discussed in this section have been summarized in Table 17.

Reactions of epoxides with n-butyllithium (n-BuLi)

n-Butyllithium, like other organolithiums, is an oligomer of varying complexity in solution. For example, in hydrocarbon solvents such as hexane or benzene, it is hexameric[166] (Fig. 66); tetrameric[166] (Fig. 67) in ether, and dimeric (solvated) in tetrahydrofuran.[167] In addition, its electron deficient nature[168] allows it to co-ordinate with Lewis bases such as ethers and amines.[169,170] with consequent depolarization to varying extents. Kinetically, its basicity increases as the aggregate size diminishes, and therefore, tetrahydrofuran is the solvent of choice for generating reactive species.

Epoxide rearrangement to allylic alcohols using n-butyllithium has been demonstrated in medium size ring epoxides. Nozaki et al.[59] reported the β-hydrogen abstraction of cis and trans-epoxycyclododecanes by n-butyllithium leading to trans allylic alcohol (Fig. 28). The attempted reactions of epoxides (2), (3), (125), (126), (154) and (155) with n-butyl

Figure 65

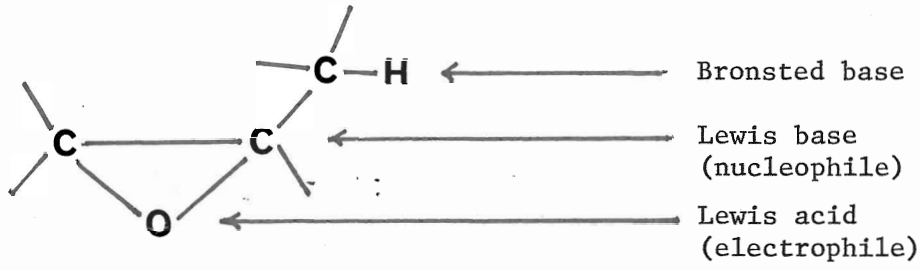
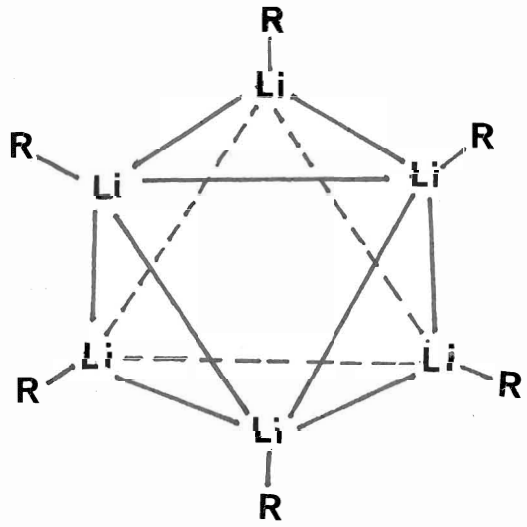
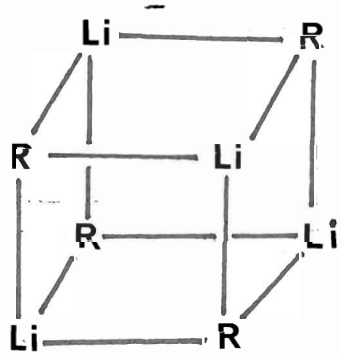


Figure 66



R = n-butyl

Figure 67



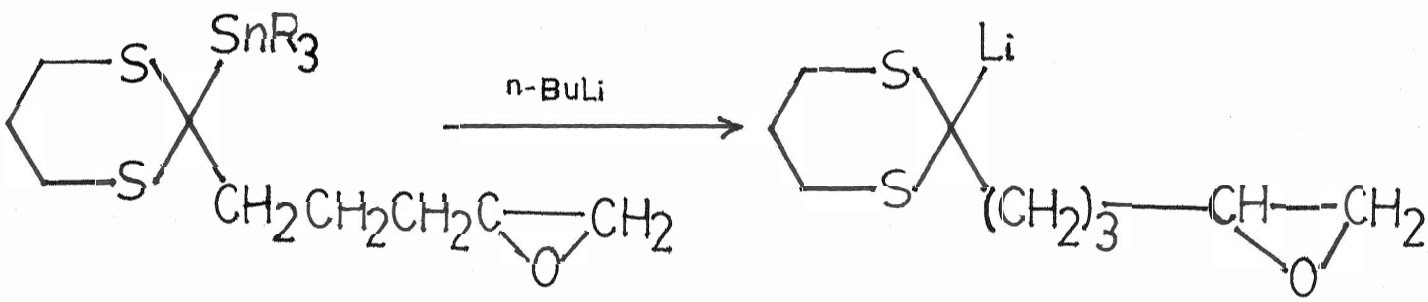
R = n-butyl

lithium for three days at room temperature afforded only the corresponding starting materials, which were identified by comparing spectral data with authentic samples.

The inert behaviour of these epoxides towards n-butyllithium may be simply due to an insufficient basic strength of n-butyllithium for bringing rearrangement of these epoxides by either α- or β-eliminations. The nucleophilic opening of these epoxides by n-butyllithium is probably prevented because of the bulkiness of the reagent and the steric crowding that will be created in products if formed. The reaction conditions adopted in the present investigation may be milder than those reported for the epoxide rearrangements with n-butyllithium and perhaps vigorous conditions might be necessary.

On the other hand, the occurrence of β-elimination in epoxycyclododecyl systems[59] (Fig. 28) may also be due to the provision of suitable conformations for reaction to occur. The medium size rings have been known[171] to exist in their several conformations, unlike those found in small ring compounds. Therefore, it may be possible that under the reaction conditions, these steroids epoxides were incapable of providing suitable conformations in the transition state for reactions to occur. However, n-butyllithium has been effectively used in certain reactions without affecting oxirane rings. Juenge and Seyferth[172] demonstrated the lithiation of dithiane derivative carrying an epoxide side chain, in trans metallation reaction (Fig. 68). The epoxide moiety remained intact[172] even though it had both α- and β-hydrogen available for α- and β-eliminations. The steric inhibition in this epoxide opening can not be considered because

Figure 68



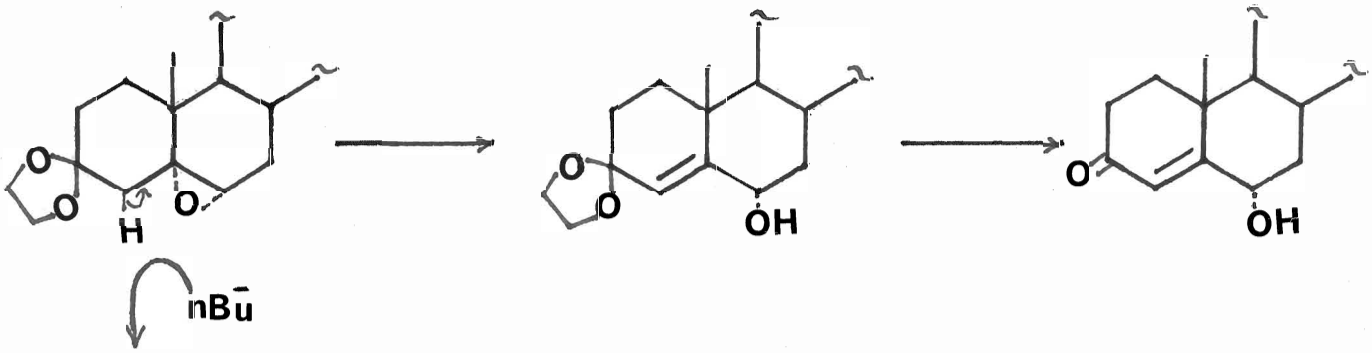
of its well separation from the dithiane ring by three methylenes. Therefore, this example[172] may be taken as the liteature precedent consistent with the unreactivity of epoxy steroids (2), (3), (125), (126), (154) and (155), towards n-butyllithium observed in the present study.

In the continuing investigation of epoxide reactions with n-butyllithium, 5α,6α-epoxycholestane-3,3-ethylenedioxy ketal (150) gave exclusively Δ^4-cholesten-3-one-6α-ol (174). Monitoring the reaction with tlc indicated the consumption of epoxide (150) in two hours even at 0°C in contrast with other epoxides without ketal moiety at C3 carbon. This suggested a strong activation provided by the ketal functionality.

The identity of the UV inactive intermediate which later on acid workup gave (174) was confirmed by isolating it in controlled isolation using pH 6.86 buffer. From ^{1}H NMR and mass spectrum, the intermediate was characterized as Δ^4-cholesten-6α-ol-3,3-ethylenedioxy ketal (173). Identification of the intermediate (173) confirmed the course of reaction, in which n-butyllithium abstracted the C4 hydrogen, followed by epoxide opening to give (173). The ketal group of (173) was then hydrolyzed on acid workup to yield (174) (Fig. 69).

Model inspection of α-epoxy ketal (150) showed that 4β-hydrogen has more or less trans arrangement with the oxirane ring. Therefore, in an anti-elimination, 4β-hydrogen will be abstracted by the base from the β-face, and will lead to the formation of (173) (Figure 70). But the 4β-hydrogen is sterically crowded by the presence of C19 angular methyl group, and therefore the anti-elimination can not be considered as the only mechanism for the formation of (174).

Figure 69



(150) (173) (174)

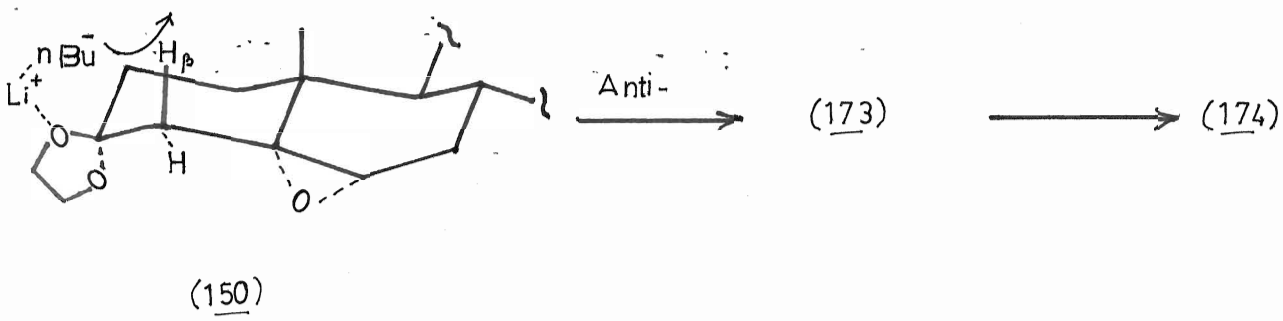
In the chairform conformation of ring A, the 4α-hydrogen does not have perfect cis-orientation with epoxygen. A slight deformation in ring A is required to bring about a cis-relation between 4α-hydrogen and the epoxide ring. This deformation may be easily effected by the co-ordination with n-butyllithium from the α-face and consequently a syn-elimination may easily be effected through a six-membered transition state (Fig. 71).

Barton et al.[42] reported base induced rearrangement of 5α,6α-epoxy-cholestan-3-one to corresponding unsaturated hydroxy ketone. Using the deuterium labelled compound, they have demonstrated that the rearrangement occurs via apparent syn-elimination[173]; only 4α-hydrogen was lost exclusively during this rearrangement (Fig. 72). Theimmel and Richbourn[62,174,175] have also reported syn elimination in cyclohexane epoxides as the preferred way of rearrangement.

In the present study, neither of the elimination mechanisms (anti- and syn-) can be distinguished, and labelling experiments will be required to make any certain decision in this regard.

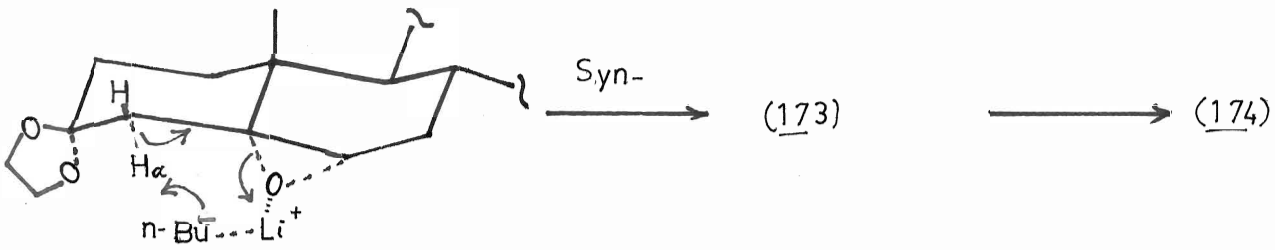
The corresponding reaction of 5β,6β-epoxycholestan-3,3-ethylenedioxy-ketal (151) with n-butyllithium under similar conditions afforded unexpected results. The reaction with β-epoxy ketal (151) was comparatively slow and resulted in the formation of two products. After chromatographic separation and repeated crystallization, two compounds were isolated and characterized as 3β-n-butylcholest-4-en-3α,5β-diol (175) and 3β-n-butyl-5β,6β-epoxycholestan-3α-ol (176). The formation of (176) as

Figure 70



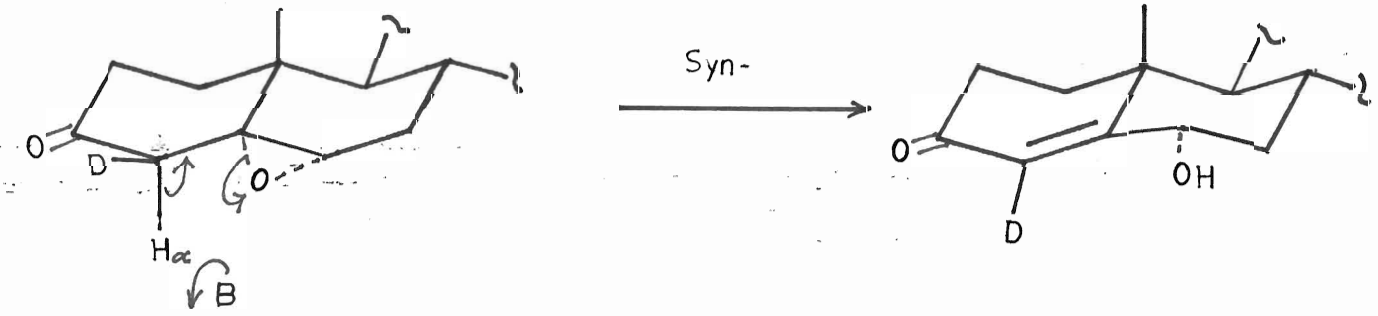
(150)

Figure 71



(150)

Figure 72



minor product (9.92%) is probably indicative of its intermediacy in conversion to (175) which was the major product (75.5%) from the reaction.

The β-stereochemistry of the n-butyl group in these two new compounds (175) and (176), is proposed on the basis of mechanistic and stereochemical considerations. This β-stereochemistry may follow from co-ordination of n-butyllithium to the β-epoxide and in fact a mixture of α- and β-butyl derivatives was obtained when the epoxide was absent. The correlation between the observed chemical shifts in ^{13}C NMR of (175) and (176), and that calculated from 1,1-alkyl substituted cyclohexanols also supported the β-orientation of n-butyl group in these two new compounds.

Several mechanisms could account for the formation of (175) and (176), however a reasonable mechanism will include (176) as an intermediate. The reagent, n-butyllithium, used in the reaction was at least in tenfold excess, therefore suggesting a comparatively slow conversion of epoxy product (176) to its diol isomer (175). This is in agreement with its molecular model inspection, showing that neither of the two C4 hydrogens has the required orientation relative to the 5β,6β-oxirane ring suitable for either anti- or syn-elimination. Another piece of evidence in this regard is the attempted reaction of 5β,6β-epoxycholestan-3α-ol (126) with n-butyllithium. Even after three days at room temperature, β-epoxide (126) showed only the development of a very minor product spot on tlc, which was not isolated. This epoxide (126) was regarded almost unreactive under these reaction conditions.

Considering β-epoxy product (176) as an intermediate on the route to the 3,6-diol isomer (175), it is reasonable to say that n-butyl group is most probably responsible for bringing a deformation in ring A and therefore promoting β-elimination from C4 carbon.

The product distribution in the reaction of (151) and n-butyllithium also suggests that in this particular example, the ketal opening by n-butyllithium is faster than β-elimination and therefore Δ^4-cholesten-3-one-6β-ol (177) was not obtained (Fig. 73). This unique behaviour of β-epoxy ketal (151) may be accounted for by its cis A/B ring junction which will offer steric hindrance to n-butyllithium attacking C4 hydrogen from either α- or β-face. This is also consistent with the predictions from model studies.

The probable mechanism for the formation of (175) and (176) from (151) is represented in Figure 74. n-Butyllithium attacks the C3 carbon from the less hindered β-face which is facilitated by lithium co-ordination with the ketal oxygen on the same side. This attack is accompanied by concurrent breaking of C3-O bond of ketal group on the α-side in S_N2 fashion followed by fragmentation to acetaldehyde (Fig. 74) to give (176).

Once the epoxy product (176) is formed, another molecule of n-butyllithium co-ordinates with epoxygen and brings the ring A in half chair conformation, which orients 4β-hydrogen cis to the epoxide. Now the 4β-hydrogen could be removed by syn-elimination leading to the formation of (175) (Fig. 75).

Nevertheless, the anti-elimination of the C4α-hydrogen, which has been well demonstrated by labelling experiments[173] in the base-catalyzed

Figure 73

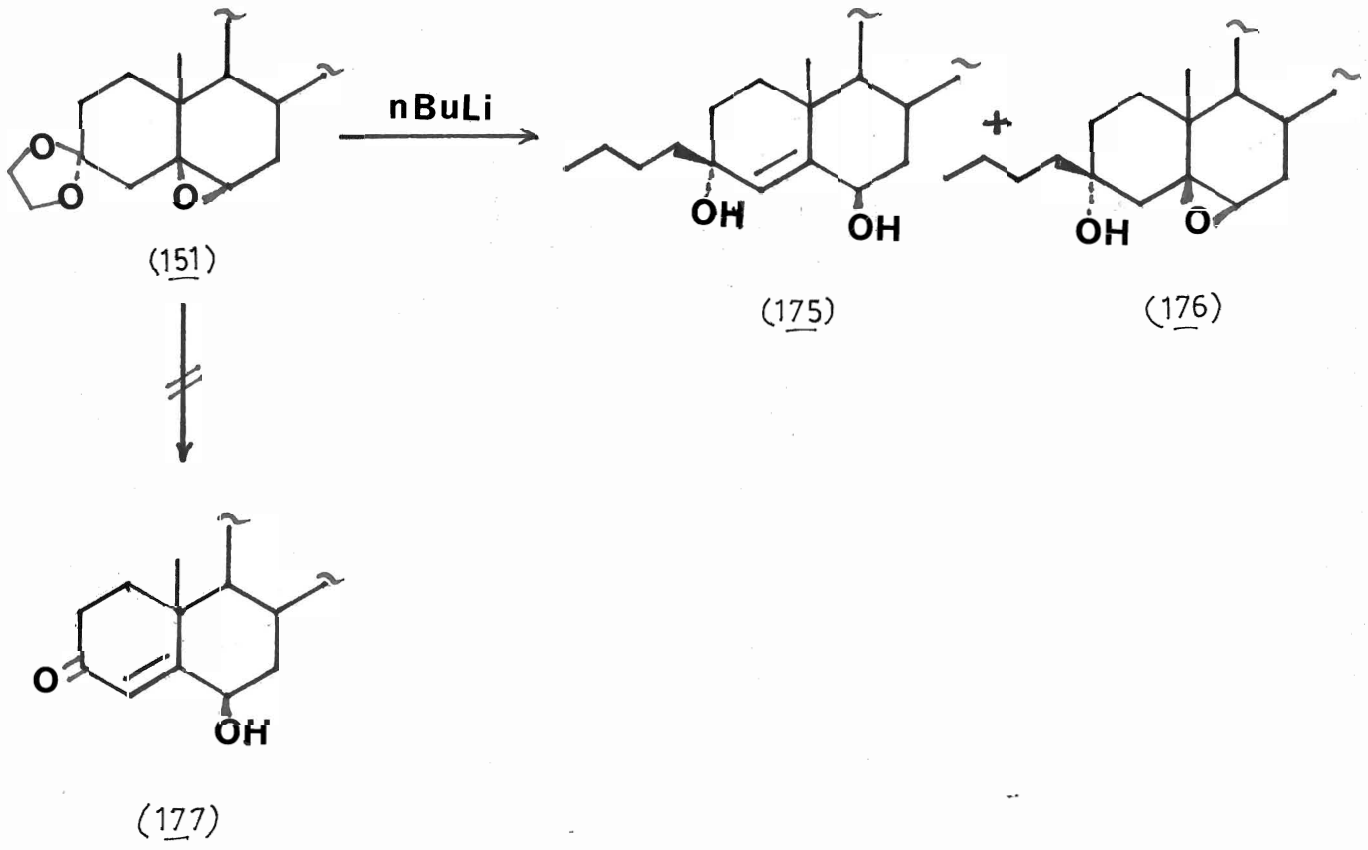
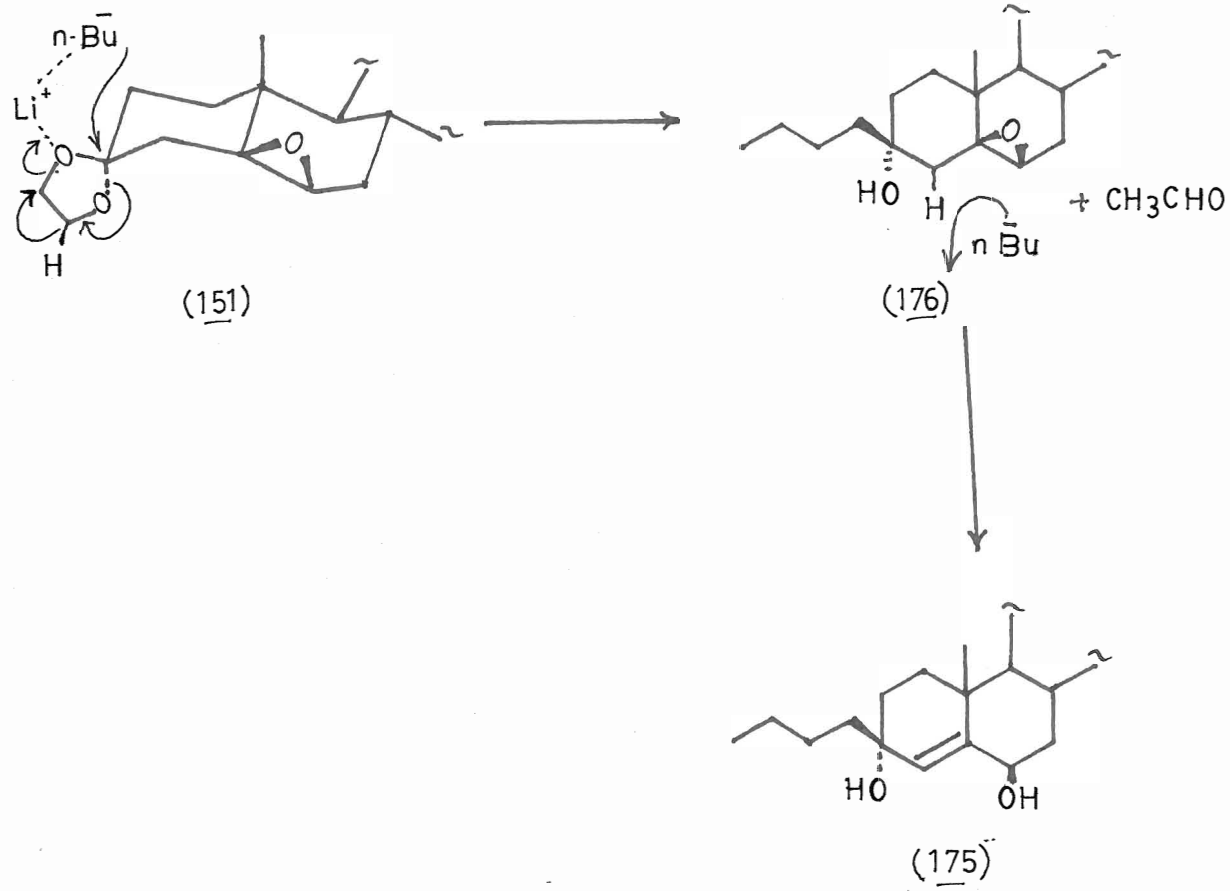
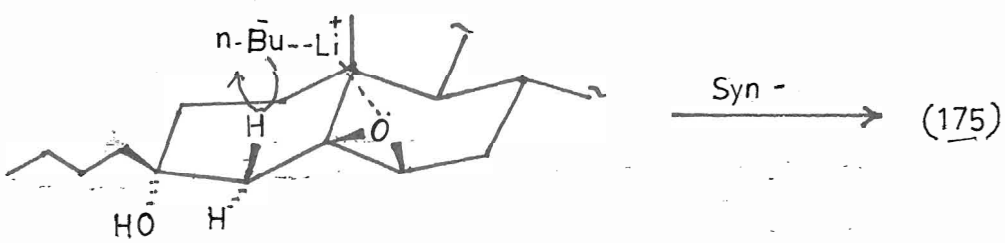


Figure 74



(151) (176) (175)

Figure 75



(175)

rearrangement of 5β,6β-epoxycholesten-3-one (Fig. 76), can not be ignored here. Therefore, in anti-elimination, stereochemical factors require the 4α-hydrogen to be removed from an axial position. Hence, the epoxide (176) must adopt the boat conformation of ring A in the transition state (Fig. 77). Nevertheless, these two eliminations can not be finally distinguished without labelling experiments.

Murai and coworkers[176] proposed a closely analogous mechanism (Fig. 79) of ketal fragmentation to acetaldehyde with n-butyllithium during the synthesis of 2-hydroxycyclobutanone derivatives (Fig. 78). Their[176] observation may be considered as a literature precedent in support of present proposed mechanism (Fig. 74) for the formation of (175).

The structure (175) was further supported by making its monoacetate derivative, 3β-n-butylcholest-4-en-3α,6β-diol-6-acetate (175A). The presence of 6-hydroxyl group was also evident from the oxidation of (175) Preparation of 3β-n-butylcholest-4-en-6-one-3α-ol (178) by both Jones and and MnO_2 oxidations was accompanied by side reactions, most probably originating from dehydration, and the product was not isolated in pure form.

Reactions of 5α-cholestane-3,3-ethylenedioxy ketal (181) with n-butyllithium and methyllithium

Having observed the very unusual fragmented opening of a ketal moiety in the β-epoxy ketal (151) by n-butyllithium, further investigations were desired in this regard. 5α-Cholestane-3,3-ethylenedioxy ketal[111] (181),

Figure 76

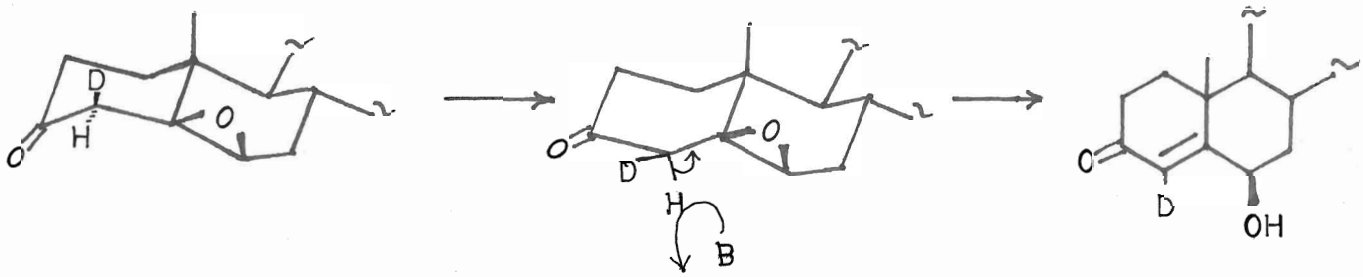


Figure 77

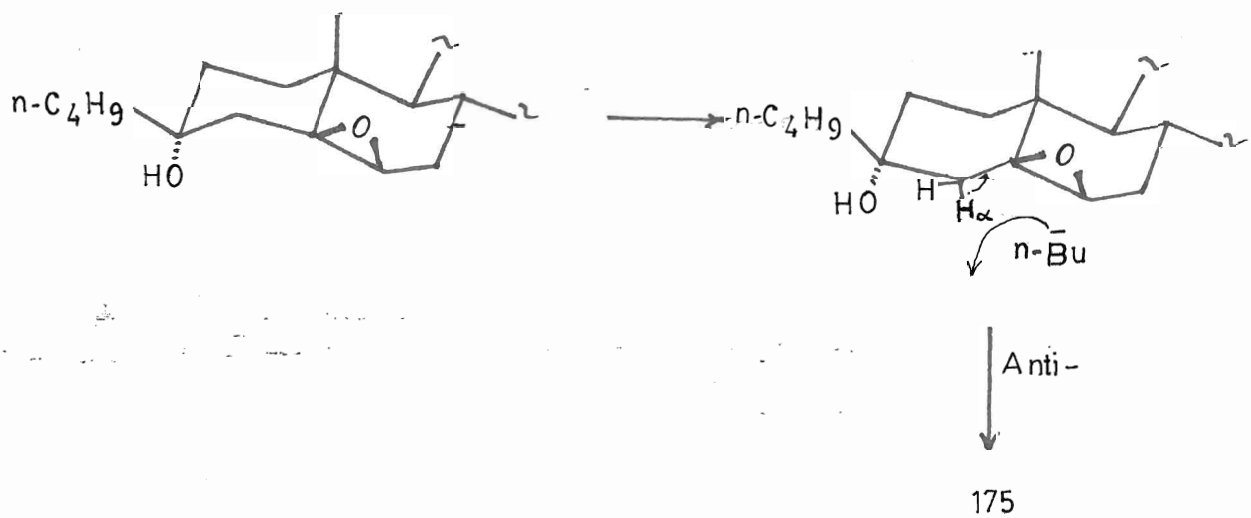


Figure 78

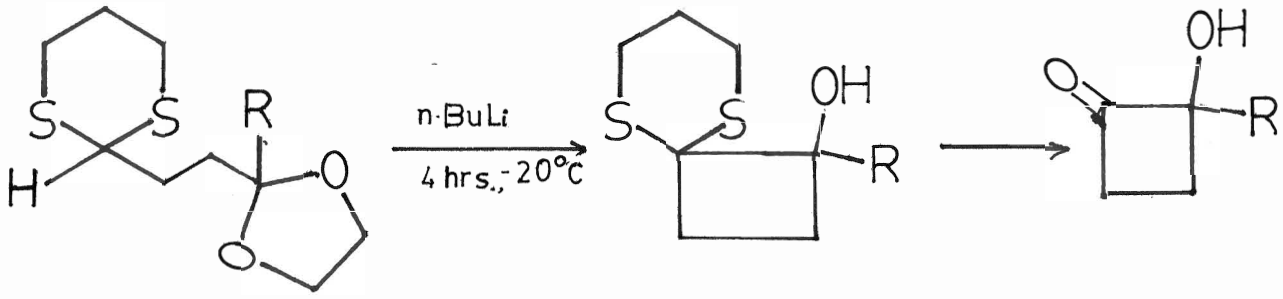
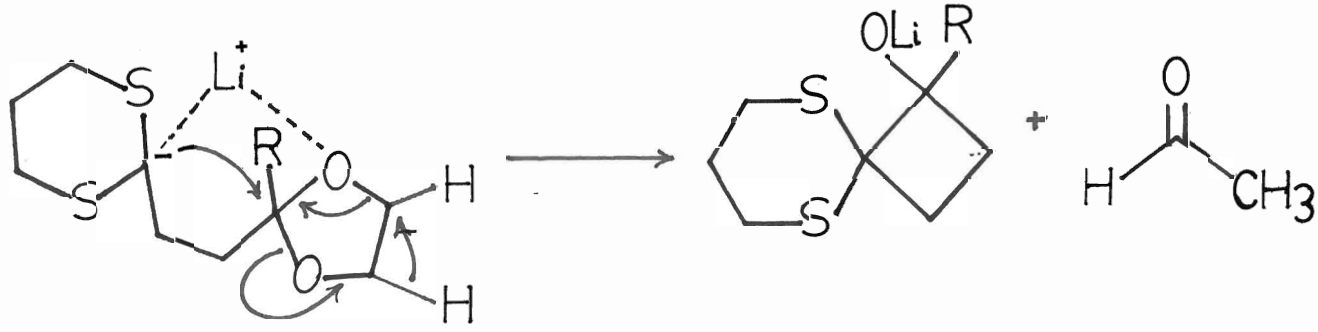
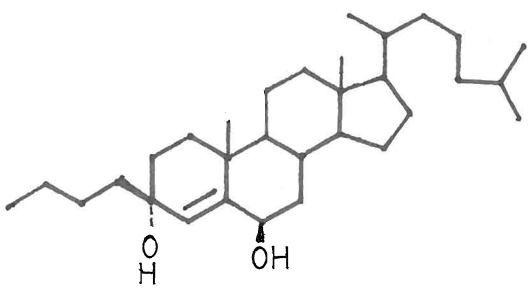
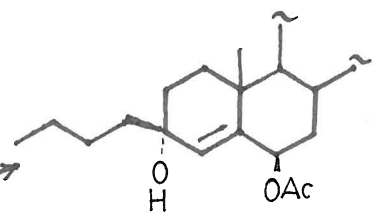


Figure 79

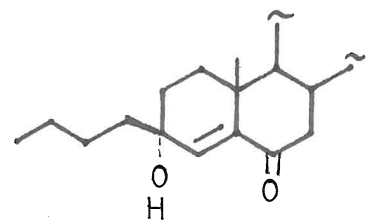




(175)



(175A)



(178)

for this purpose, was easily prepared by ketalizing 5α-cholestan-3-one (180), which in turn was obtained by Jones oxidation of 5α-cholestan-3β-ol (179) (Fig. 80).

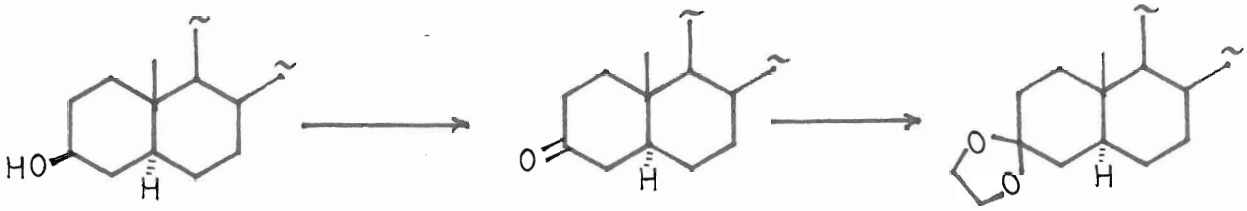
The reaction of (181) with n-butyllithium by stirring at room temperature for 2 hours gave a mixture of two products. After chromatographic separation on neutral alumina, two new compounds, 3β-n-butyl-5α-cholestan-3α-ol (182) (62.1%) and 3α-n-butyl-5α-cholestan-3β-ol (183) (17.5%) were isolated and characterized by spectral means. This reaction provided further evidence of the ketal opening capability of n-butyllithium and therefore supported the findings with 5β,6β,epoxycholestan-3,3-ethylenedioxy ketal (151).

As expected, on conformational grounds (trans A/B ring function), the n-butyllithium can attack the ketal group of (181) from both the α- and β-faces. The preferential attack from the less hindered β-face (78.0%) was as expected. This is also explainable in terms of product stability, because in the major product (182), n-butyl group occupies more stable equatorial position. Attack from the α-face is more hindered and the product (183) has greater steric crowding because of the axial disposition of the n-butyl group. This is consistent with the formation of (183) as a minor product (17.5%).

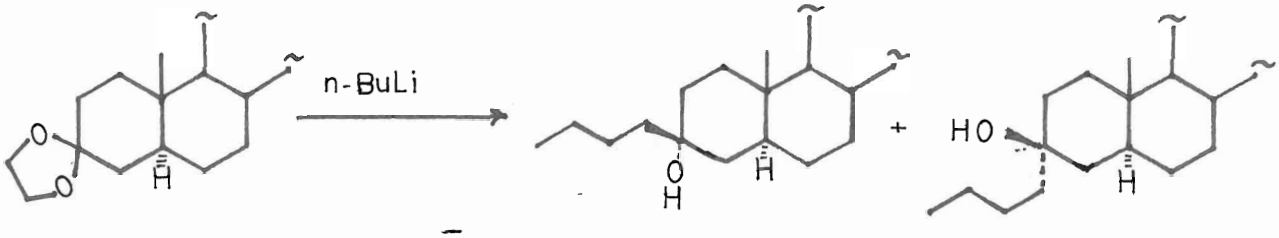
A mechanism similar to that presented in Figure 74 can also be proposed for the formation of the above two isomeric alcohols (182) and (183) (Fig. 81).

In the continuing investigations on ketal opening by alkyllithium, only 60% of (181) was reacted with methyllithium (MeLi) even after 4 days

Figure 80

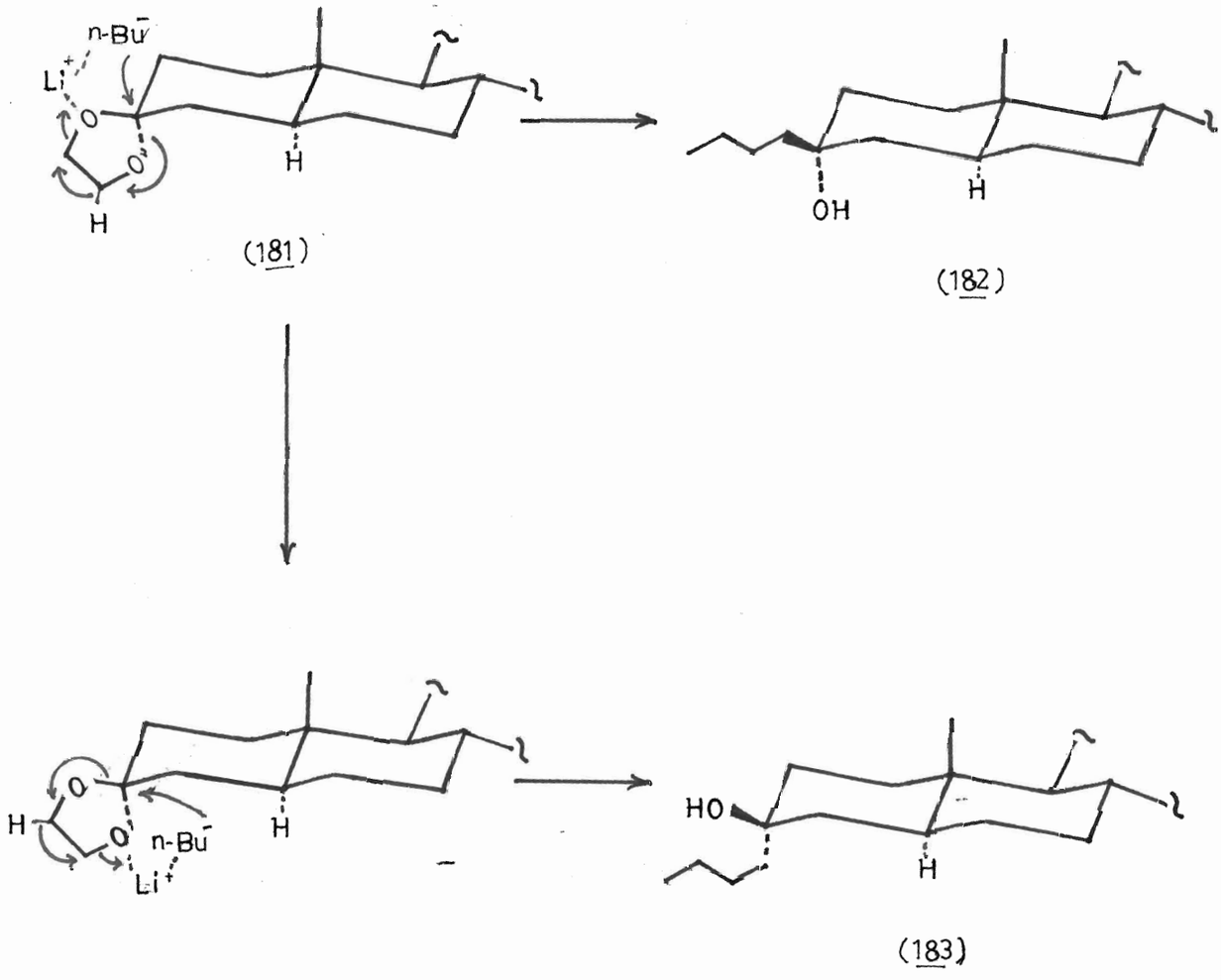


(179) (180) (181)



(181) (182) (183)

Figure 81



(181) (182)

(183)

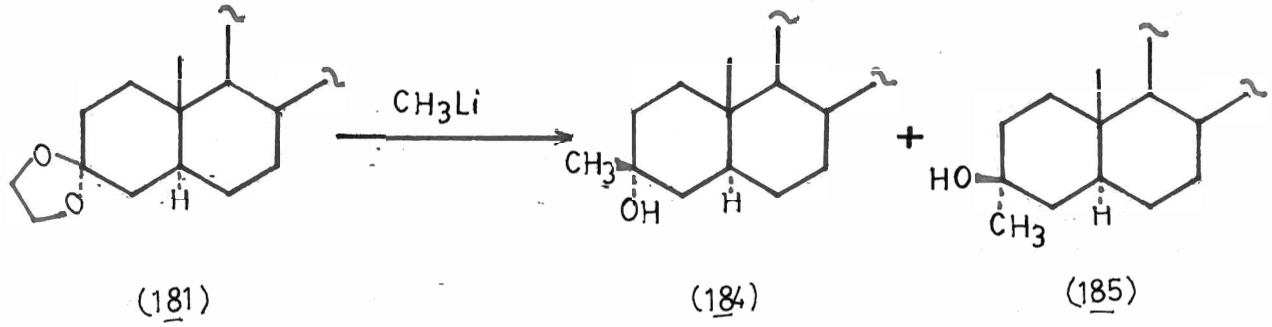
stirring at room temperature. This poor reactivity with methyllithium may be accounted for by its comparatively weaker basic strength in fragmentation of ketal group. Along with 40% unreacted starting material (181), two isomeric alcohols, namely, 3β-methyl-5α-cholestan-3β-ol (184) (32.1%) and 3α-methyl-5α-cholestan-3β-ol (185) (27.81%) were isolated and identified by spectral means.

Since the methyllithium is very small compared to n-butyllithium, it can attack almost equally from both the α- and β-sides. The relative ratio of α- and β-alcohols (1.15:1) indicated only a slight preference of β-side attack on ketal (181). Barton et al.[117] reported the formation of these isomeric alcohols (α and β) in the ratio of 1.32:1 from the reaction of methylmagnesium iodide on cholestan-3-one (180), also indicating a little preference for an equatorial methyl group (184).

A mechanism similar to that with n-butyllithium can also be presented for the formation of (184) and (185) from (181) (Fig. 82).

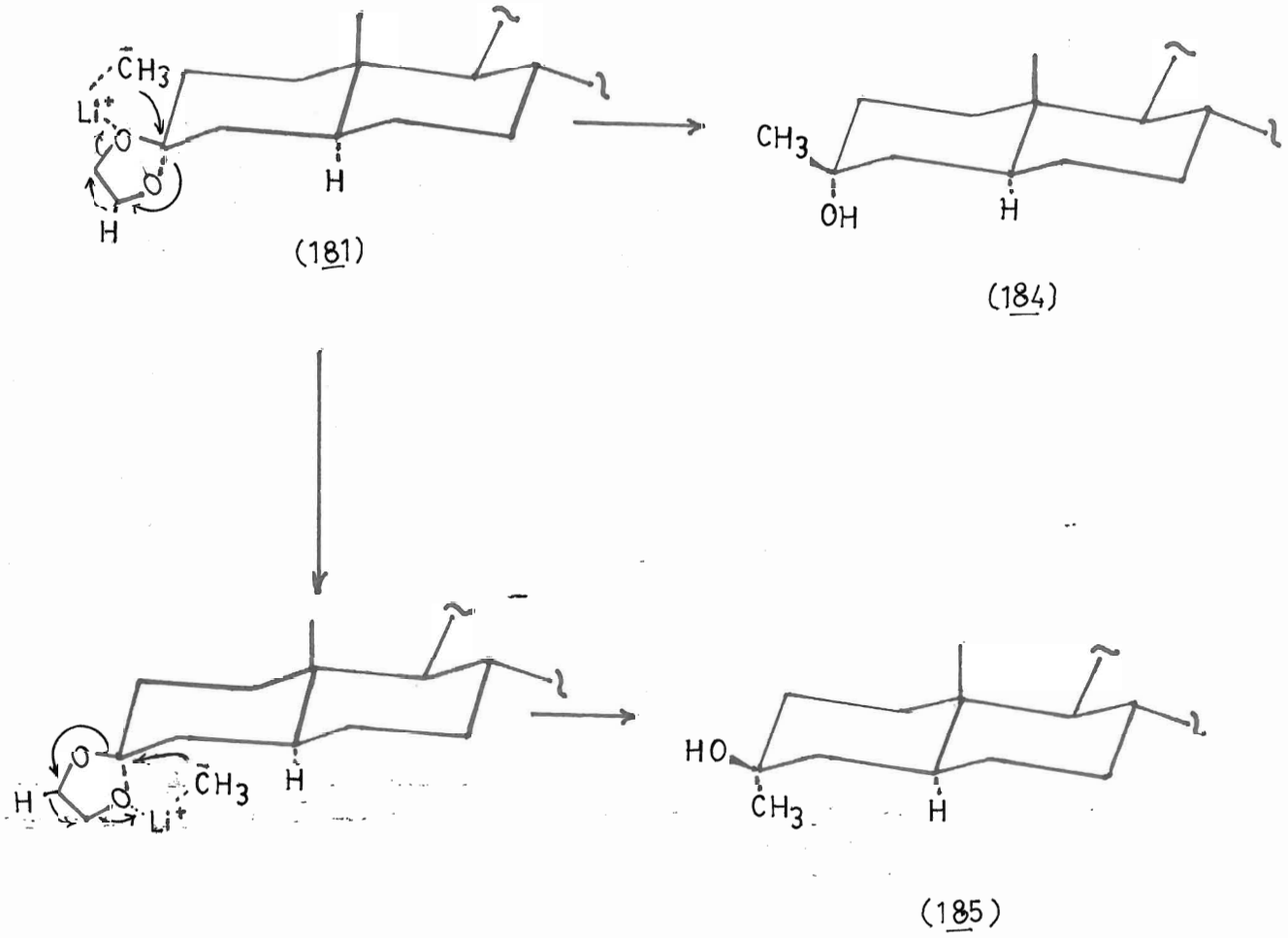
Sokolova et al.[177] have reported the epoxide opening of the 16α,17α-epoxypregnane derivative with methyllithium without affecting the ketal group present in the molecule (Fig. 83). However, Nedelec and Gase[178] observed the ketal opening promoted by methyllithium in a reaction with the epoxy ketal (186) in a different fashion (Fig. 84). The product obtained from this reaction was an aromatic ether.

The recognition of the synthetic utility of ketal opening, as observed in the present study, by allyllithiums, will certainly require some more specialized investigations in this area and therefore bringing its application in general organic syntheses.



(181) (184) (185)

Figure 82



(181) (184) (185)

Figure 83

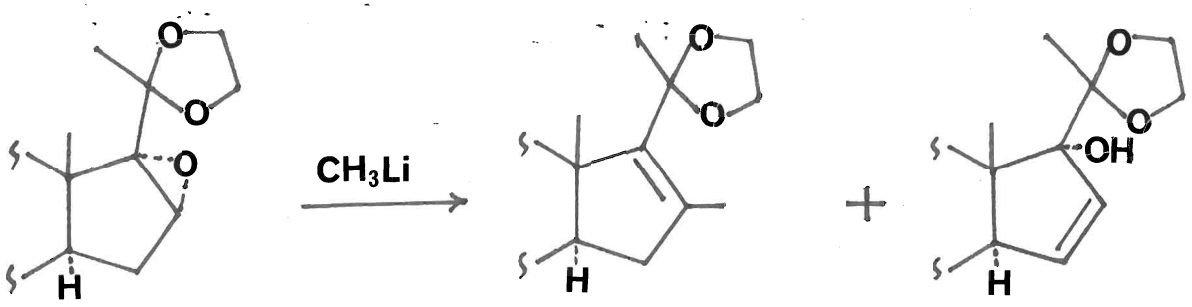
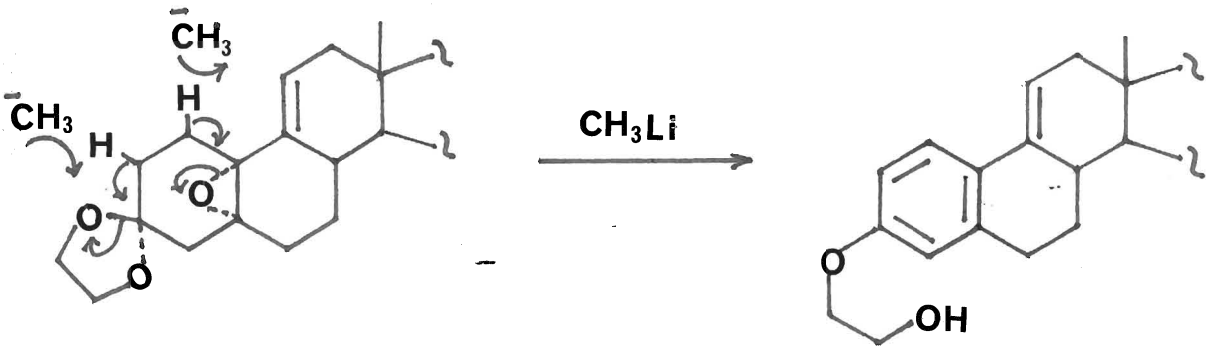


Figure 84



(186)

Attempted reactions of epoxides with potassium t-butoxide

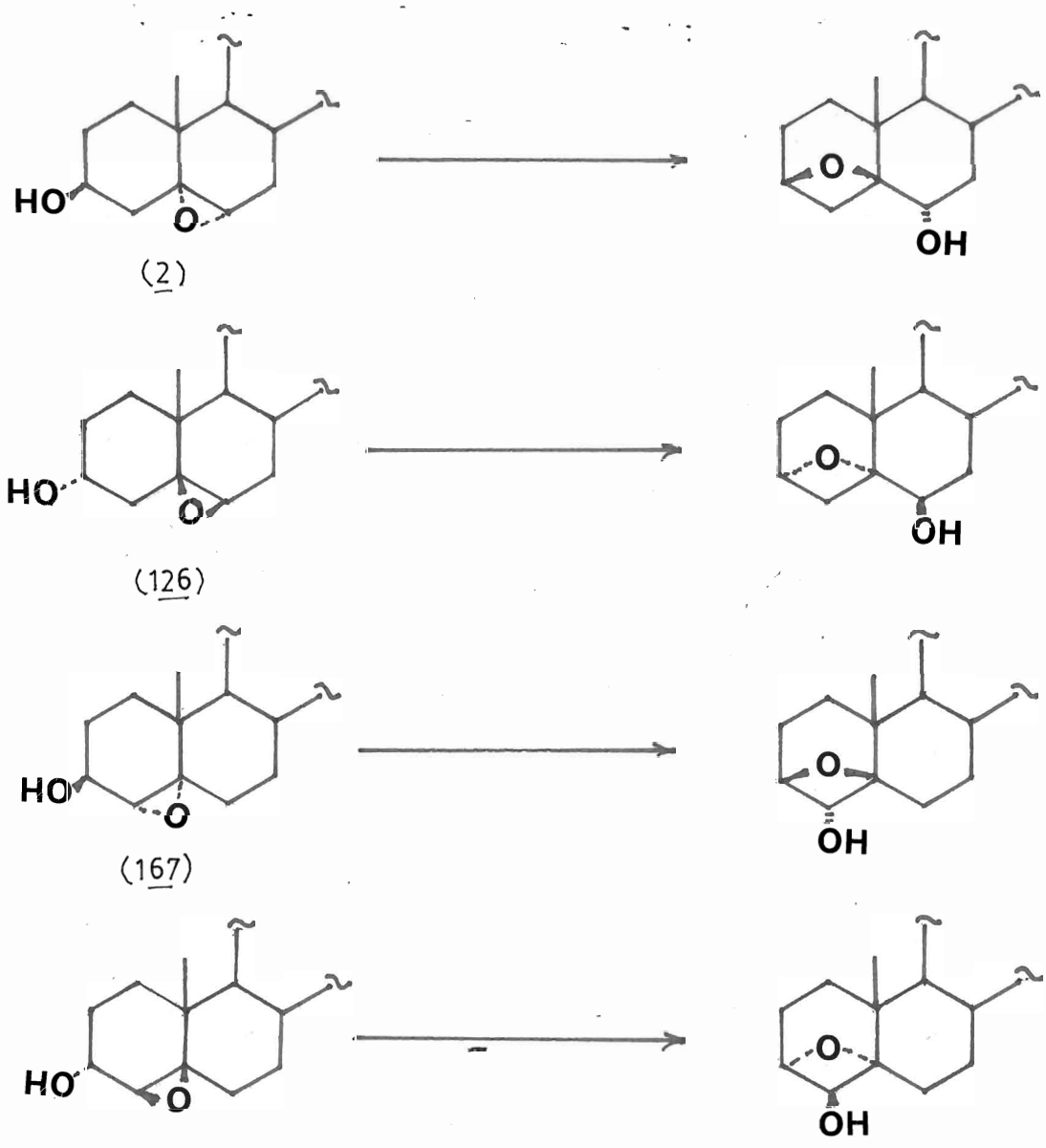
Because of its poor nucleophilic nature and very strong basic character, potassium t-butoxide has been used extensively in organic syntheses. Its capability of promoting rearrangement of epoxides to the corresponding allylic alcohols,[50,51] via β-elimination, has also been demonstrated (Fig. 23).

Attempted reactions of all the 4,5 and 5,6-epoxides with potassium t-butoxide even with refluxing for three days, afforded only the corresonding starting epoxides. It was expected that epoxides having a 3-hydroxyl group of opposite stereochemistry to the epoxide ring may lead to the formation of the corresponding oxetanes. For instance, epoxides (2), (126), (167) and (169) may be expected to give the corresponding oxetanes (Fig. 85). From the unreactivity of these epoxides with n-butyllithium, it was thought that the co-ordination of lithium cation with the oxygen of the hydroxyl group may be strong and therefore make the latter unavailable for further attack of the oxirane ring. The hope that K^+ will reduce this complication of strong metal-oxygen co-ordination, reactions were carried out using potassium t-butoxide which also resulted in failure.

Attempted reactions of 5,6-epoxides with lithium diisopropylamide (LDA)

Some 28 years have elapsed since the topic of metallation reaction was reviewed by Gilman and Morton.[179] The intervening years, especially the last decade, have been notable for intensive exploration in this area,

Figure 85



leading to the discovery of many organolithium reagents[170,180,181] among these lithium diisopropylamide (LDA) is notable. The usefulness of lithium diisopropylamide has been widely recognised[182-186] in various aspects of synthetic organic chemistry.[187-189]

Because of some technical difficulties encountered, the commercially available powder of lithium diisopropylamide was not used. It was prepared,[190,191] *in situ*, from diisopropylamine and methyllithium at -10°C in THF, and was immediately used under a dry nitrogen atmosphere. The LDA so prepared was tested by conducting its known reaction[115] with o-toluic acid; the dianion generated was reacted with n-bromobutane. The GC-mass spectrum of the crude product indicated over 95% conversion to o-n-pentyl-benzoic acid (Fig. 86).

Attempted reactions of all the 5,6-epoxides (2), (3), (125), (126), (150), (151), (154) and (155), resulted only in failure even after conducting reactions for three days. During these reactions, the temperature was not allowed to rise above -5°C, because LDA can deprotonate THF at 0°C (Fig. 87).

The two isopropyl groups in LDA are most probably making it unable, due to steric crowding, to abstract hydrogens of required geometry in these steroidal epoxides. Although it has been used effectively in open chain, small and less sterically crowded medium size ring epoxides,[76,78] Vandewalle *et al.*[192] observed its very low yield performance when compared to lithium diethyl amide during the synthesis of prostaglandins. Vigorous conditions, on the other hand, can not be applied using LDA, as it starts deprotonating THF (solvent)[193] even at 0°C (Fig. 87).

Figure 86

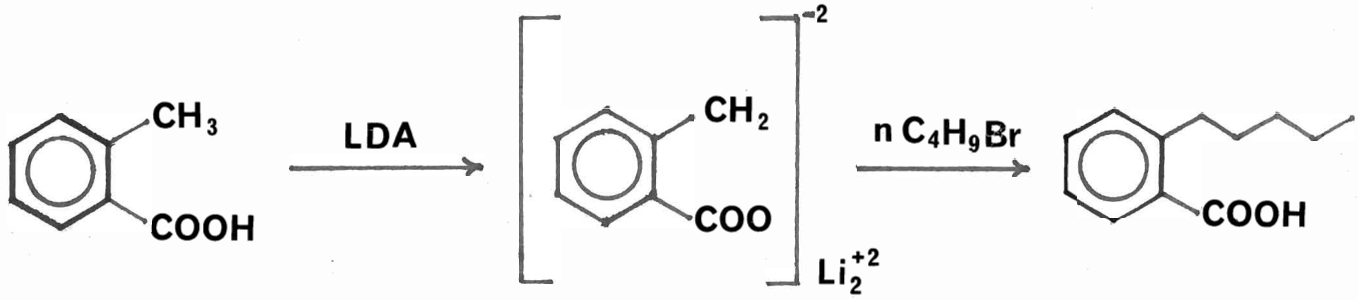
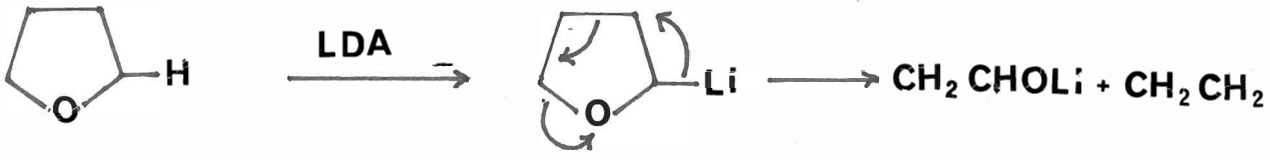


Figure 87



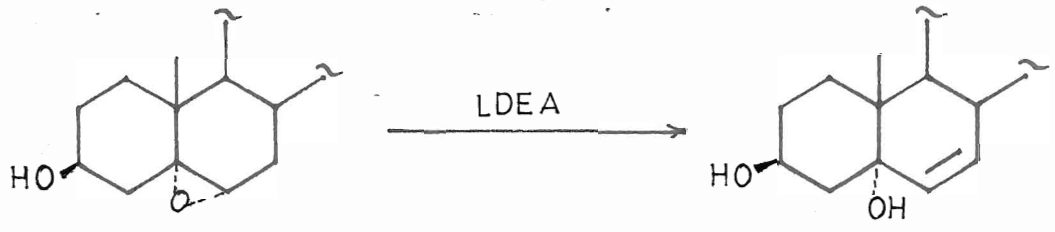
Reactions of 5,6-epoxides with lithium diethylamide (LDEA)

Failures with LDA led us to use smaller lithium alkylamides. Lithium diethylamide-promoted rearrangement of epoxides have been known since 1951[63] and can take several routes depending upon the structure of the epoxide.

Treatment of 5α,6α-epoxycholestan-3β-ol (2) with LDEA under reflux conditions for 24 hours afforded Δ^6-cholesten-3β,5α-diol[118] (187) as the only product which was isolated in 56.5% yield after crystallization. The formation of C6-C7 double bond in the product (187) suggests the rearrangement of the epoxide (2) has occurred selectively by β-elimination from the C7 carbon.

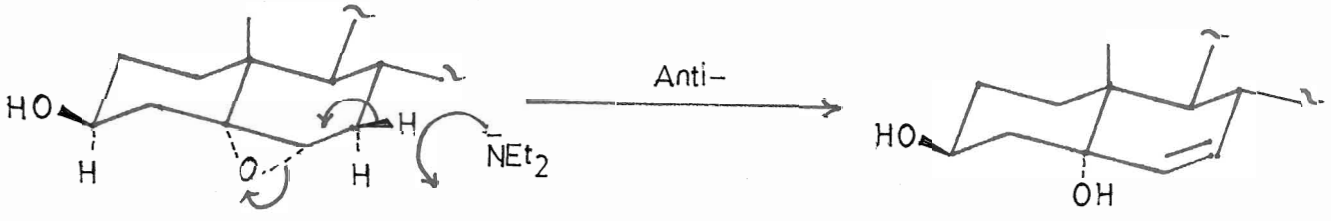
Although there were two hydrogens, 4β- and 7β-hydrogen, in perfect orientation for anti-elimination, only the C7 hydrogen was abstracted selectively by LDEA. The removal of 4β-hydrogen is inhibited most probably on steric and to some extent on electrostatic grounds. Under the reaction conditions, 3β-hydroxyl group will carry a negative charge and may prevent the approach of Et_2N^- from the β-face to abstract the 4β-hydrogen.

A syn-elimination of the 7α-hydrogen, however, may seem unlikely as it does not have a cis-geometry with respect to the α-oxirane ring (2). Nevertheless, this can be achieved by deforming ring B, which may be achieved by co-ordination with LDEA from the α-face (Fig. 90). The syn-elimination has been demonstrated by labelling experiments, as the preferred pathway in cyclohexane oxides.[174] Since an anti-elimination of the 7β-hydrogen will not require any conformational change, it may be the preferred pathway in the formation of (180) (Fig. 88). The rigidity of the steroidal



(2) (186)

Figure 88



(2) (186)

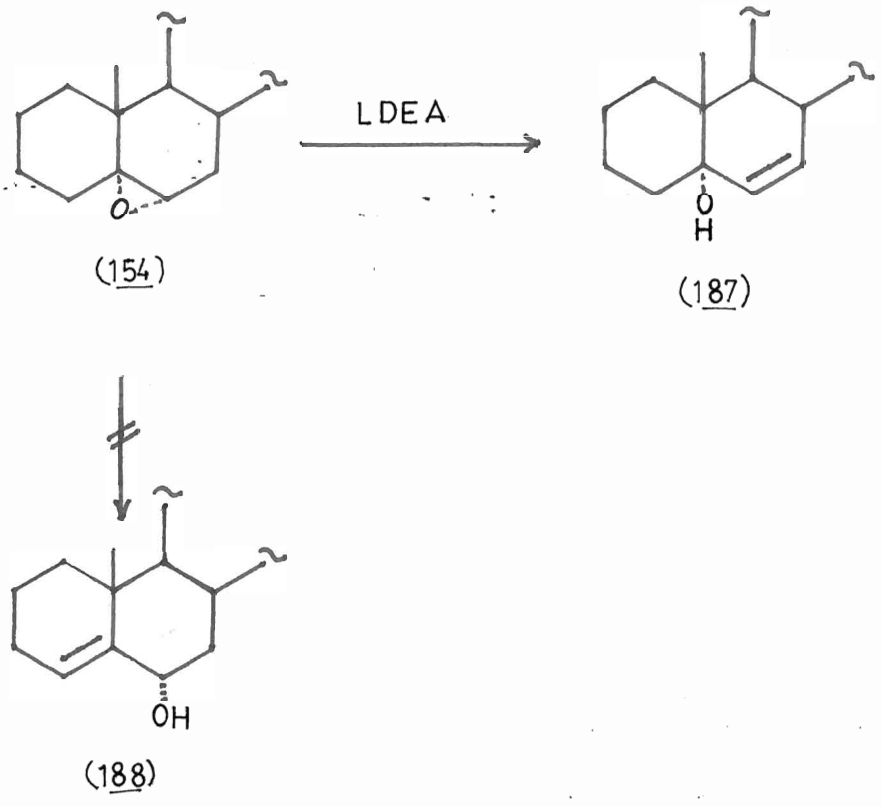
framework may dictate this deviation from the normal behaviour of cyclohexene oxides.[174]

If 3β-hydroxyl group in (2) was an additional factor contributing towards the inhibition of C4 hydrogen removal, its absence in (154) should remove this hindrance. Therefore, a statistical distribution of allylic alcohols (187) and (188) was predicted.

Treatment of 5α,6α-epoxycholestane (154) with LDEA unexpectedly yielded only a single allylic alcohol, Δ^6-cholestene-5α-ol (187) in 72.3% isolated yield. Moreover, the reaction was much faster compared to epoxides (2), and was completed in only one hour. This suggests that the presence of 3β-hydroxyl group in (2) has some long range deactivating effect on C7 hydrogen. The exact nature of this long range effect can not be explained on the basis of available data, but presumably the creation of a negative charge on the 3β-hydroxyl group brings some conformational or electrostatic change in the molecule which deactivates the C7 hydrogen. This consequently slows down the rate of rearrangement to allylic alcohol (186).

The new allylic alcohol, Δ^6-cholesten-5α-ol (187) obtained from the rearrangement of (154) has been characterized by spectral means.

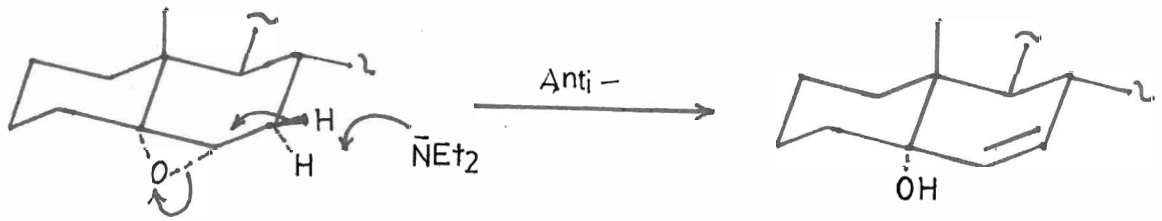
Formation of (187) as the sole product from the epoxide (154) now invalidates the steric reasoning proposed for reaction of 5α,6α-epoxycholestene (2). From stereochemical considerations, as revealed by the molecular model of (154), the allylic alcohol (187) may be formed by anti-elimination of the 7β-hydrogen (Fig. 89). However, the syn-elimination may also be effected by bringing desirable conformational change in ring



(154) (187)

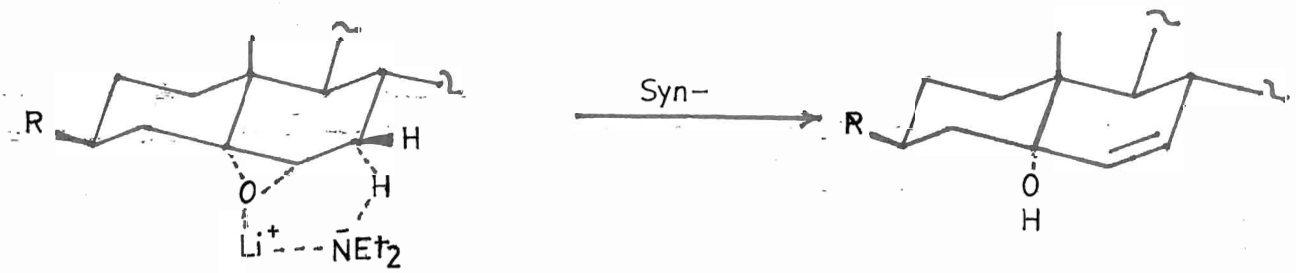
(188)

Figure 89



(154) (187)

Figure 90



(154) R = H

(2) R = OH

(187) R = H

(186) R = H

B. The LDEA in this β-elimination will co-ordinate from α-side and may bring the desired conformational variation in B to eliminate 7α-hydrogen through a six-membered transition state (Fig. 90). However, these two elimination mechanisms can not be absolutely distinguished without labelling experiments.

Exclusive β-elimination from C7 carbon yielding allylic alcohols (186) and (187) represents high regiospecificity and maybe also high stereospecificity (by the removal of similar hydrogens). Therefore, these two base-catalyzed reactions are of considerable synthetic importance for the preparation of these two allylic alcohols.

The reaction of 5β,6β-epoxycholestane-3β-ol (3) with LDEA, on the other hand, was complicated. This reaction was considerably slower than that of the α-epoxide (2) and required longer reaction time even in twenty fold excess of the reagent.

The product with highest R_f value in the reaction mixture was highly retained on a neutral alumina column. It was identified as Δ^6-cholesten-3β,5β-diol[120] (189) isolated in 33.2% yield. The second product isolated in 16.6% yield was identified as Δ^4-cholesten-3β,6β-diol (190). A white solid of high melting point (above 300°C), which separated from the crude extract on standing was not identified because of its insolubility in many solvents.

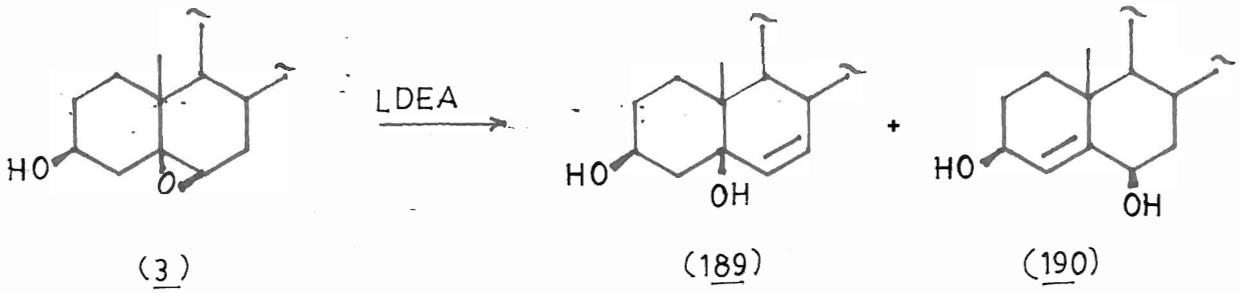
The lithium diethylamide induced rearrangement of cholesterol-β-epoxide (3) is most facile by β-elimination of hydrogen from the C7 carbon, resulting in the formation of allylic alcohol (189) as the major product. The 7α-hydrogen in (3) has a perfect trans stereochemistry with respect

to 5,6β-oxirane ring, whereas the 7β-hydrogen has a syn-clinal arrangement (vicinal angle about 90°). This suggests that the preferred pathway for the formation of (189) may be by anti-elimination of the 7α-hydrogen (Fig. 91).

The chair form conformation of ring A in (3) will not permit the β-elimination of any of the two C4 hydrogen atoms, unless the removal of C4 hydrogen is entirely independent of epoxide opening. In order to meet stereoelectronic requirements for syn-elimination of 4β-hydrogen, the ring A must adopt the half chair conformation in the transition state (191). Now the LDEA can co-ordinate from the β-face and can remove 4β-hydrogen via a six-membered transition state (Fig. 91). If 4α-hydrogen is removed in anti-elimination towards the formation of (190) now needed to assume a boat conformation.

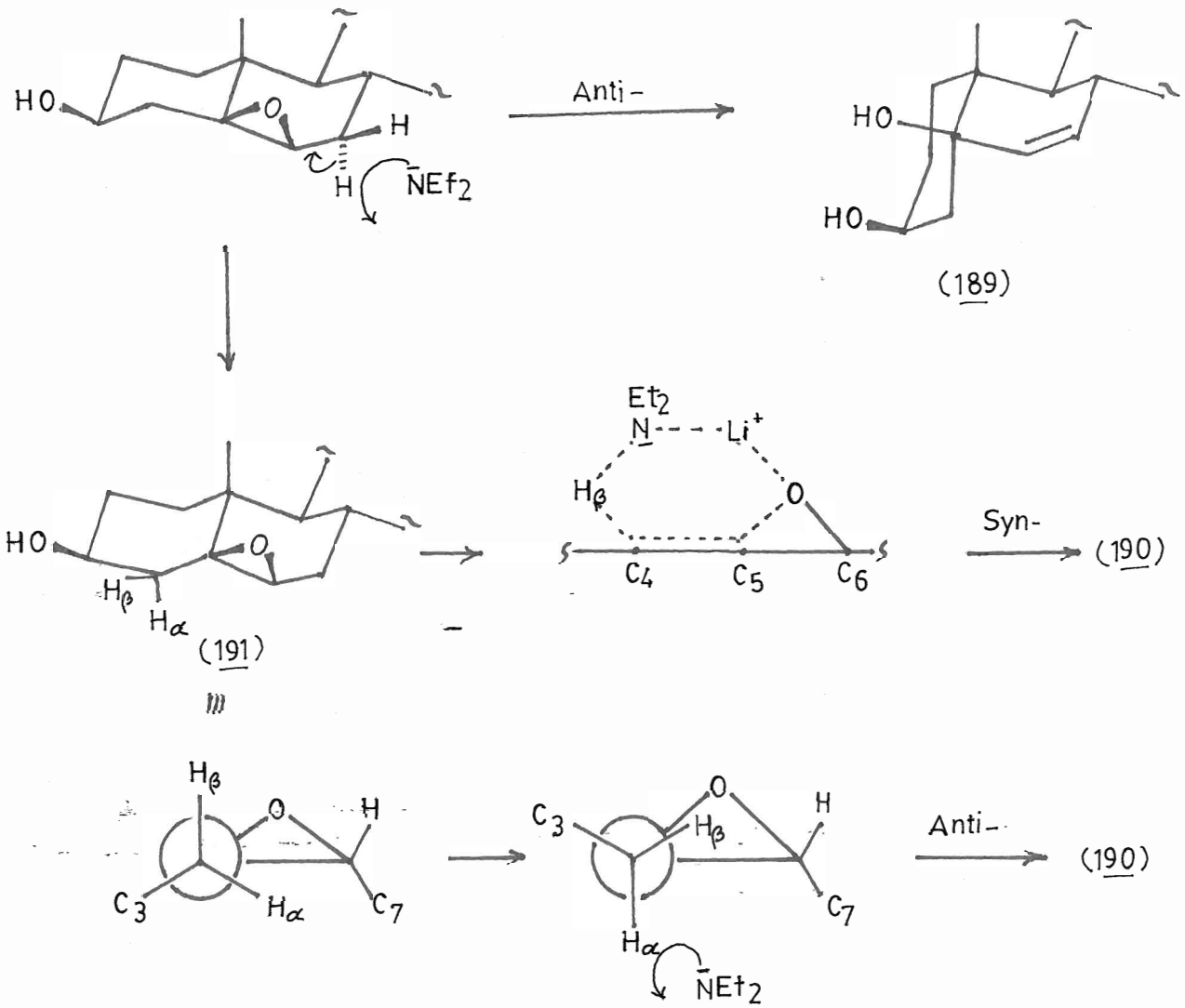
The change in the conformation of ring A from the more stable chair form to less favorable half-chair or boat forms will require activation energy. The transition states (191) and (192) will be crowded and strained. These two factors are presumably decreasing the deprotonation from C4 carbon and therefore the allylic alcohol (190) was formed as the minor product.

Treatment of 5β,6β-epoxycholestane (155) with LDEA resulted in the formation of about 5% (indicated by ^{1}H NMR) of Δ^{4}-cholesten-6β-ol (193)[103] after three days of reflux. The formation of allylic alcohol (193) could be accomplished by either anti- or syn-elimination of C4 hydrogen as proposed for the formation of (190) (Fig. 91).



(3) (189) (190)

Figure 91



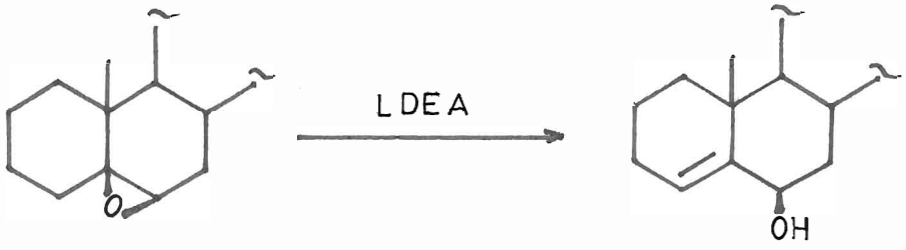
These observations on the base-catalyzed rearrangements of β-epoxides (3) and (155) suggest that the presence of 3β-hydroxyl group in 5,6β-epoxides has an activating effect on the β-elimination from the C7 carbon.

Attempted reaction of 5β,6β-epoxycholestan-3α-ol (126) with LDEA resulted in failure, although 7α-hydrogen atom was available for anti-elimination. This suggested that the presence of 3α-hydroxyl group has a very strong deactivating effect on the C7 hydrogen.

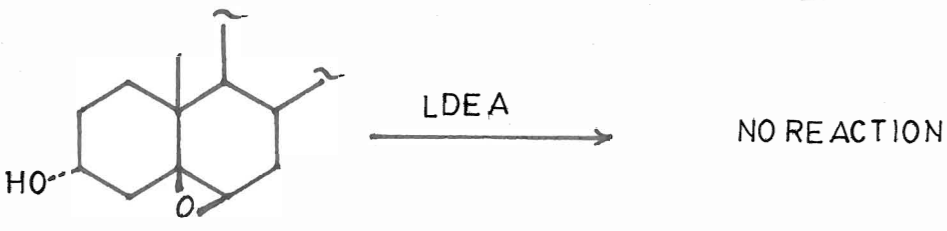
In contrast to the above, treatment of 5α,6α-epoxycholestan-3α-ol (125) with LDEA under reflux conditions for three days afforded two allylic alcohols along with unreacted starting material. The re-isolation of starting material (72.5%) clearly indicates the slow rate of rearrangements to corresponding allylic alcohols. The two allylic alcohols produced in this base-catalyzed rearrangement are Δ^4-cholesten-3α,6α-diol (194)[194] and a Δ^6-cholesten-3α,5α-diol (195). Both the allylic alcohols were formed in almost equal yields (18.0%).

The rearrangement of the epoxide (125) to allylic alcohols (194) and (195) has obviously occurred by the β-elimination of hydrogens from the C4 and the C7 carbons. The model inspection of epoxide (125) reveals that 4β- and 7β-hydrogen atoms has trans-stereochemistry with respect to 5,6α-oxirane ring. Abstraction of 7β-hydrogen by LDEA satisfies the stereochemical requirements for anti- rearrangement to Δ^6-cholesten-3α,5α-diol (195).

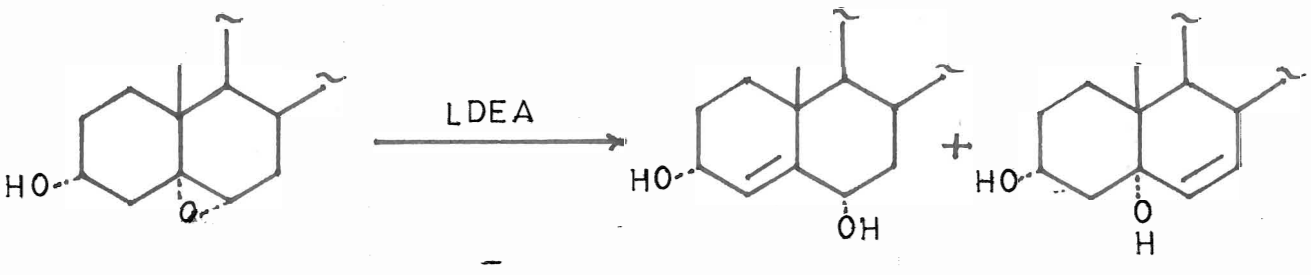
Similarly, anti- rearrangement of epoxide (125) to Δ^4-cholesten-3α,6α-diol (194) would require the β-elimination of 4β-hydrogen (Fig. 92).



(155) (193)

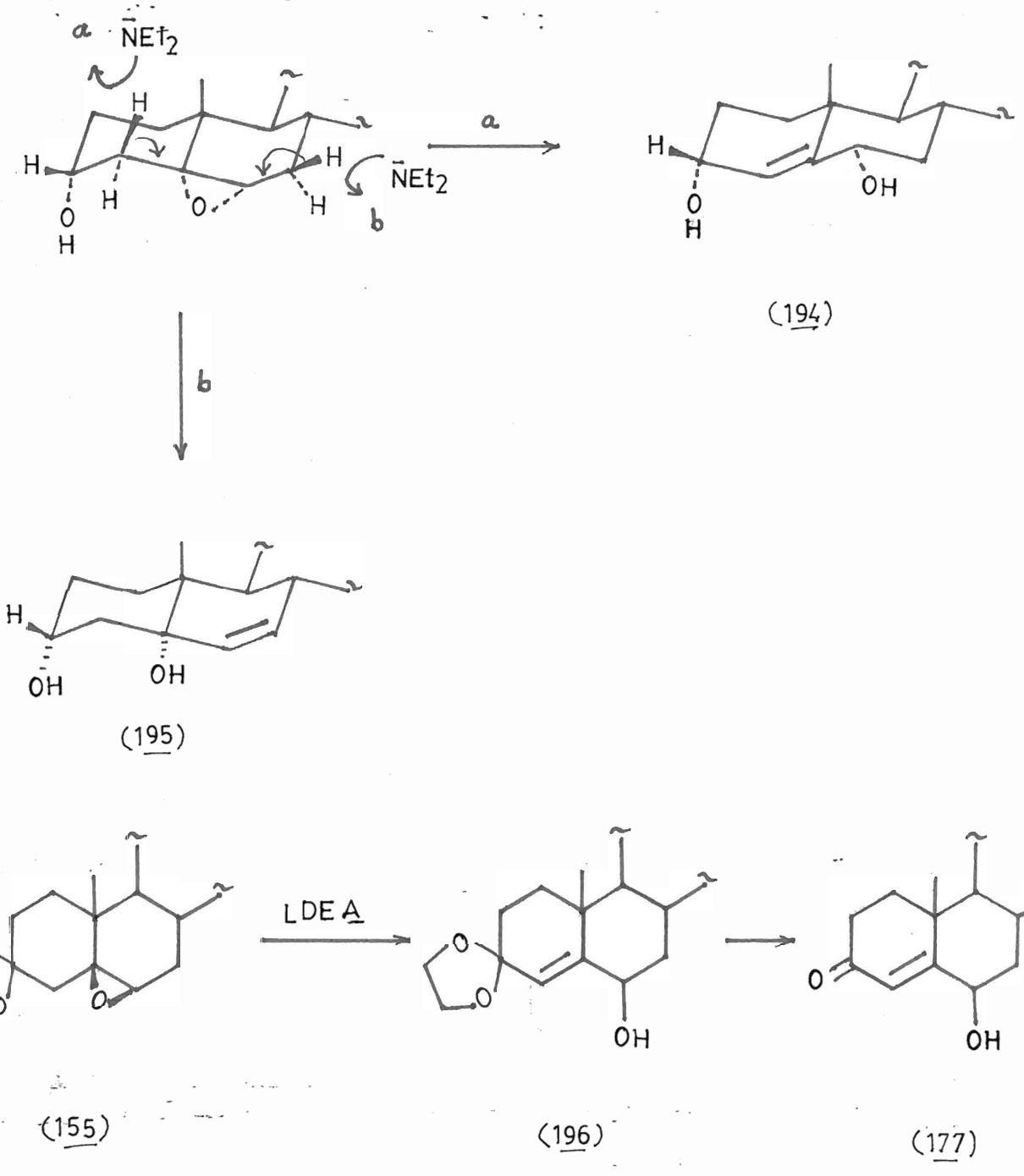


(126)



(125) (194) (195)

Figure 92



(194)

(195)

(155)

(196)

(177)

A syn-elimination of 4α-hydrogen is probably discouraged because of the axial hydroxyl group at C3 carbon, however it can not be neglected.

After having studied the LDEA-promoted allylic rearrangements of 5,6-epoxides without and with hydroxyl group at C3 carbon, corresponding epoxides carrying C3 ketal group were selected for further investigations.

In this sequence, treatment of 5β,6β-epoxycholestan-3,3-ethylenedioxy ketal (151) afforded a complex mixture. The only compound isolated in pure form was Δ^4-cholesten-3-one-6β-ol (177) (30%). The formation of Δ^4-cholesten-3-one-6β-ol (177) itself explains the intermediacy of Δ^4-cholesten-6β-ol-3,3-ethylenedioxy ketal (196) which during workup would be hydrolyzed rapidly to the conjugated ketone (177).

The formation of (177) can be explained only by deprotonation from C4 carbon. However, in the chairform conformation of ring A, neither of the two hydrogens at C4 carbon fulfills stereoelectronic requirements for either syn- or anti-elimination. For anti-elimination of 4α-hydrogen will require a transition state (197) with ring A in boat form.[173] This conformational change brings 4α-hydrogen in axial position for elimination, The ring A of (151) will be required to adopt boat conformation to bring 4β-hydrogen atom cis to 5,6β-epoxide ring (Fig. 93).

Alternatively, the proton removal may preceed the opening of the epoxide ring in a stepwise mechanism, analogous to that proposed by Barton and Houminer[42] (Fig. 94A).

The corresponding reaction of 5α,6α-epoxycholestan-3,3-ethylenedioxy ketal (150) seemed to proceed in a similar way by β-elimination from C4 carbon giving rise to Δ^4-cholesten-3-one-6α-ol (174). In careful workup

Figure 93

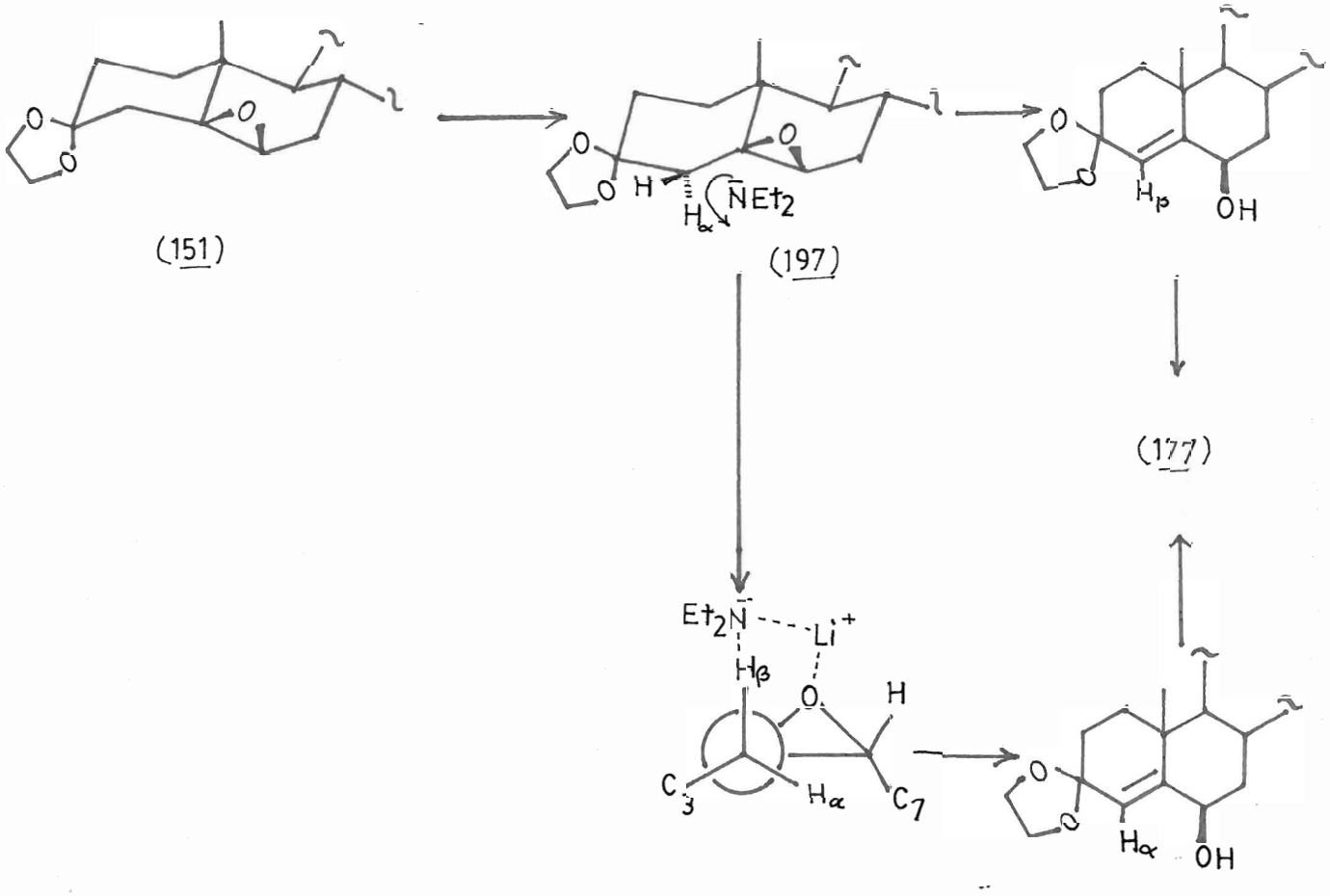
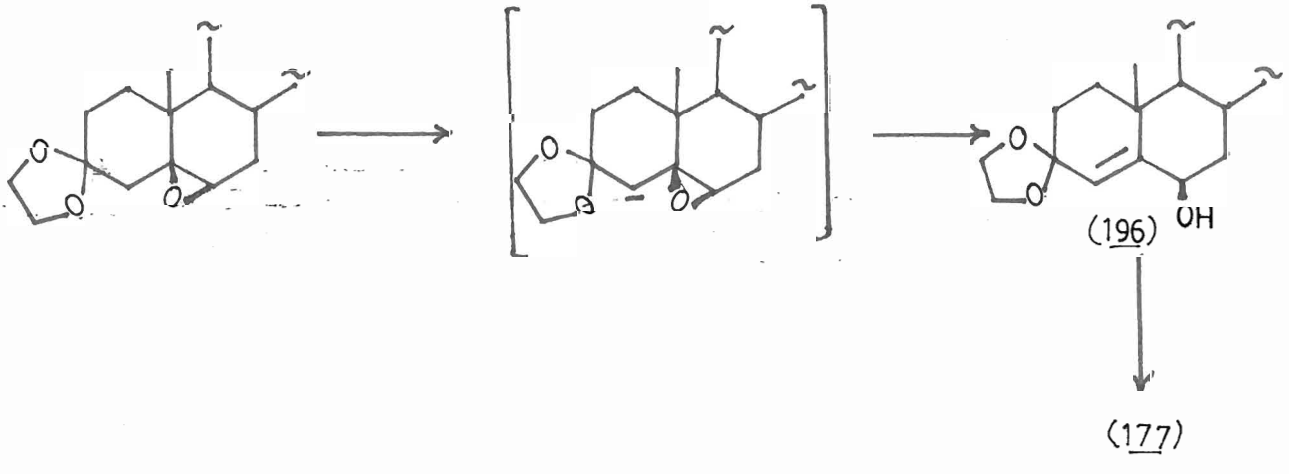


Figure 94A



using pH 6.86 buffer, the intermediate Δ^4-cholesten-6α-ol-3,3-ethylenedioxy ketal (173) was isolated with slight contamination by (174). The chromatographic purification made the crude product more complex, and only 60% of (174) was isolated.

From stereoelectronic considerations, the 4β-hydrogen has perfect orientation for anti-elimination. If it is operating during the rearrangement, then ring A does not require any conformational change. The LDEA can co-ordinate with equatorial oxygen of the ketal group and therefore directs diethylamide anion to abstract 4β-hydrogen (Fig. 94B).

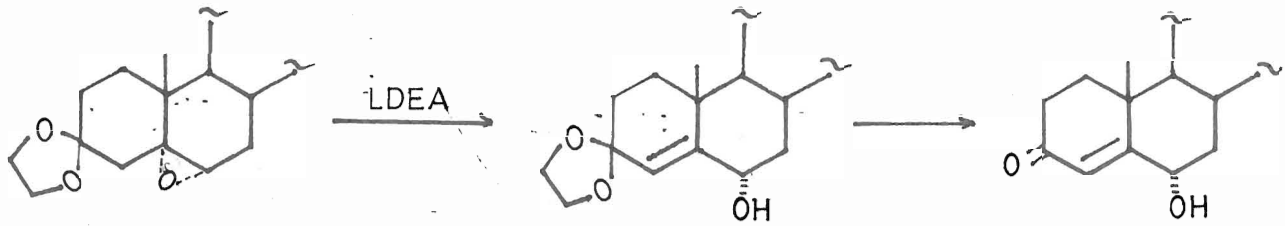
If it is assumed that 4α-hydrogen was removed by syn-elimination, as shown by Mare and Wilson[173] in case of 5α,6α-epoxycholestan-3-one, then the ring A of (150) needs to acquire boat conformation. This conformational change will orient the 4α-hydrogen in axial position and satisfies the stereoelectronic requirements for syn-elimination (Fig. 94B).

However, without labelling experiments it is not possible to distinguish between these two possible mechanisms.

Lithium diethylamide promoted rearrangements of 4,5-epoxides

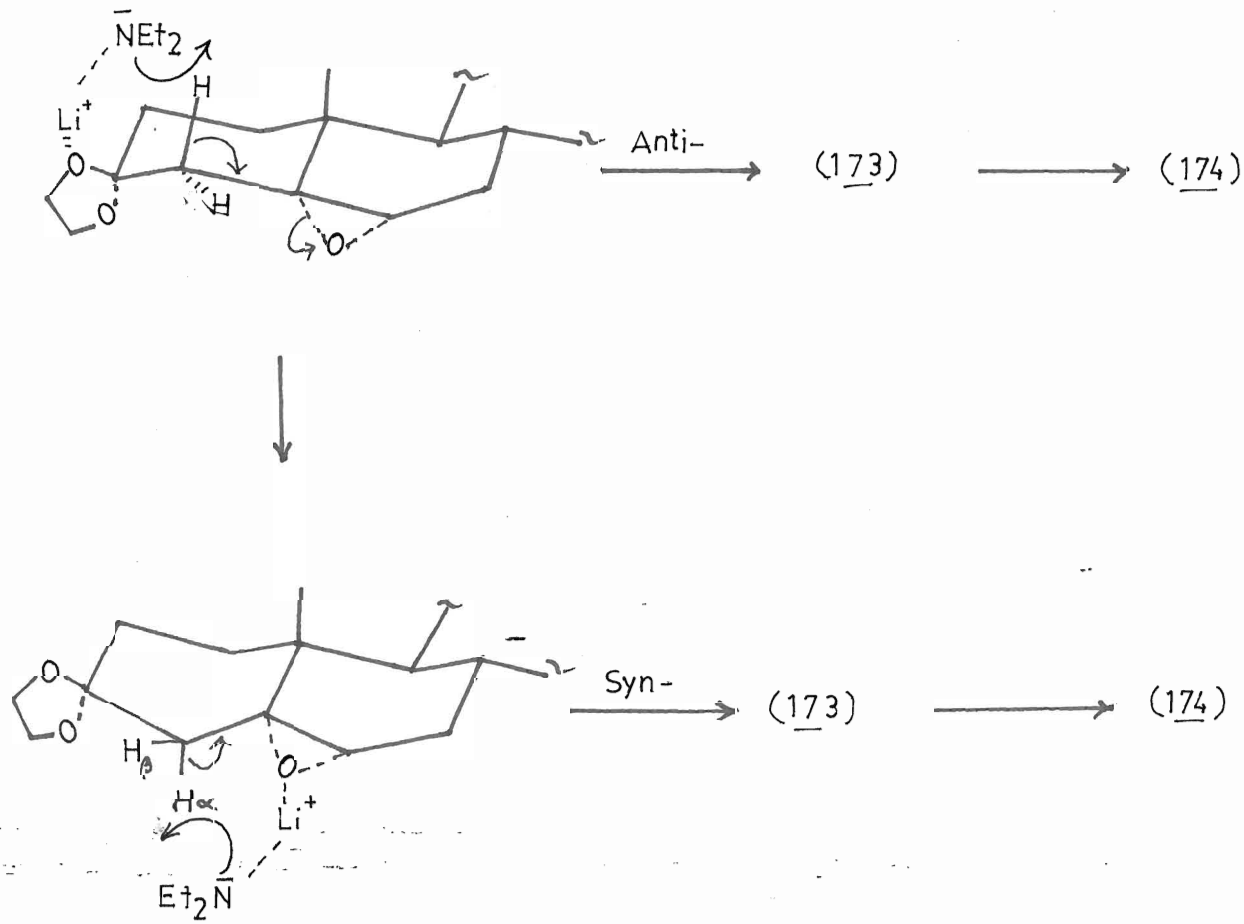
Having observed the behaviour of 5,6-epoxides towards LDEA, investigations in the corresponding reactions of 4,5-epoxides were extended.

An attempted reaction of 4β,5β-epoxycholestan-3β-ol (164) with LDEA resulted only in the re-isolation of starting material. This finding is consistent with the conclusion drawn from the molecular model of the epoxide (164). Both the methylene hydrogens at C6 carbon have such orientation neither suitable for syn- nor for anti-elimination.



(150) (173) (174)

Figure 94B



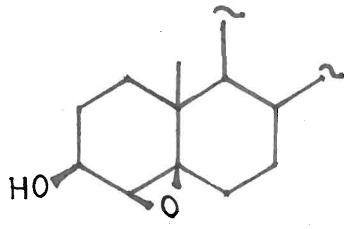
Only the 3α-hydrogen atom has trans-relationship with oxirane ring, but chemical analogy supports that the removal of this hydrogen will be exceedingly difficult. This is because the 3β hydroxyl group in basic medium carries a negative charge and the removal of 3α-hydrogen will generate another negative charge in the vicinity. The species (198) with two adjacent negative charges is highly unlikely to be formed and therefore, no reaction occurred as expected.

The failure observed during the corresponding attempted reaction of 4β,5β-epoxycholestan-3α-ol (169) is simply due to unavailability of hydrogen atom either at C3 or at C6 carbon which can meet the stereoelectronic requirements for β-elimination.

On the contrary, the corresponding reaction of 4β,5β-epoxycholestane (161) afforded two allylic products. The major product isolated in 60% yield was identified as Δ^3-cholesten-5β-ol (199)[103] and the minor product with a higher R_f value as Δ^5-cholestan-4β-ol (200)[103] (30%).

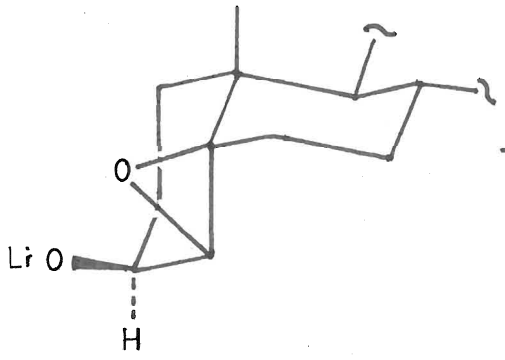
As evident from the molecular models of epoxide (161), the 3α-hydrogen atom has trans-disposition with respect to oxirane ring. This hydrogen may easily be removed in an anti-elimination process and consequently yielding the major allylic alcohol (199) (Fig. 95).

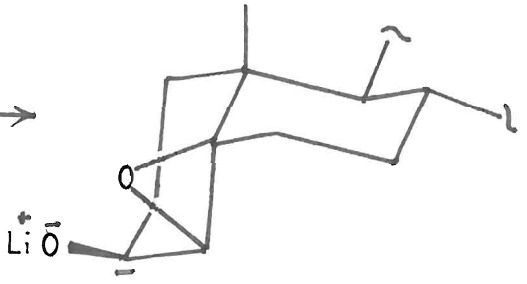
On the other hand, formation of Δ^5-cholesten-3β-ol (200) will require the removal of the C6 hydrogen. But in chair conformation of ring B, neither of two C6 hydrogens has the proper stereochemistry for E_2 eliminations. In order to explain the origin of (200) in the reaction, ring B must undergo conformational change to assume boat form in the transition state (201). This change will orient the 6α-hydrogen atom in



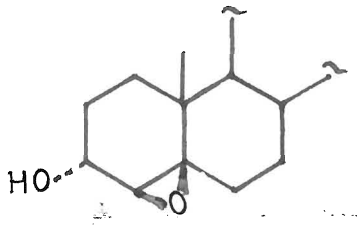
LDEA → NO REACTION

(164)



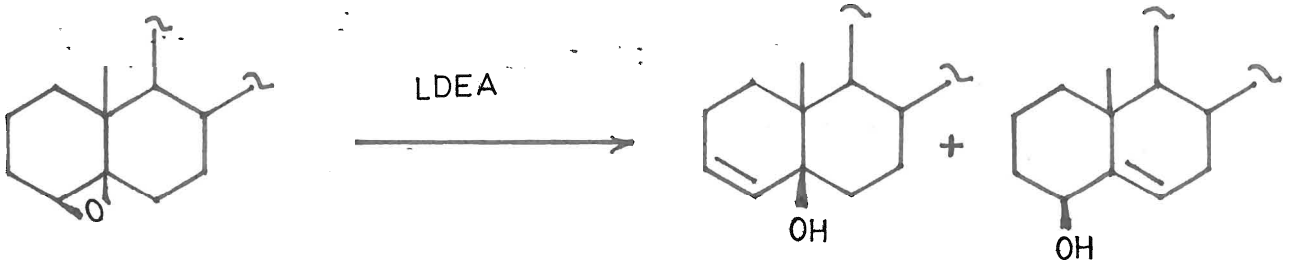


(198)



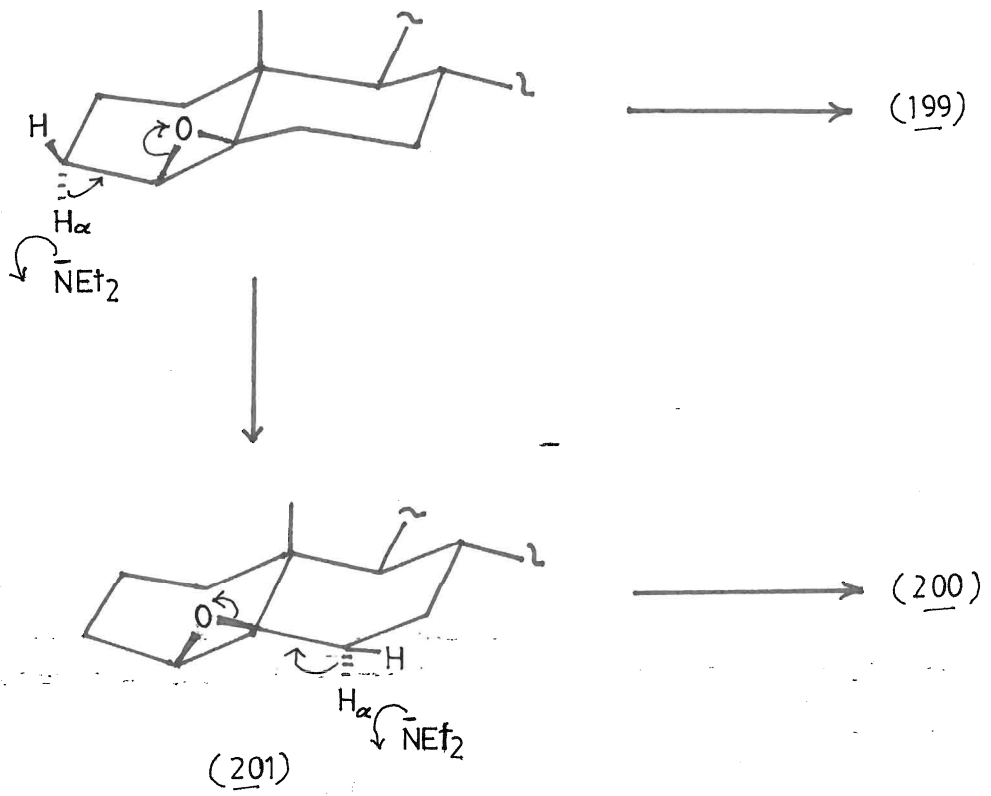
NO REACTION

(169)



(161) (199) (200)

Figure 95



(201)

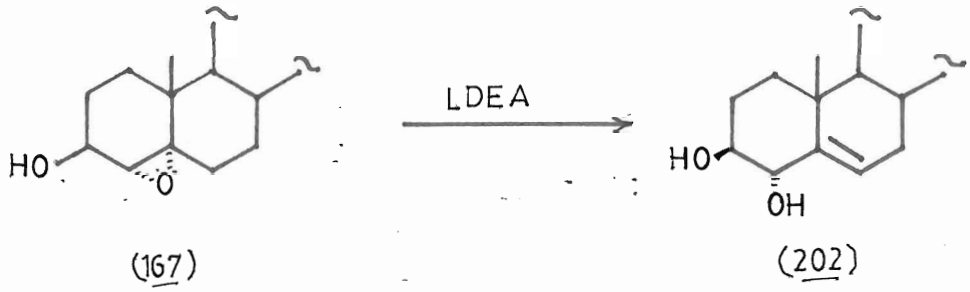
axial position suitable for anti-elimination (Fig. 95). This conformational change, although it can explain the formation of (200), will require some activation energy and also the transition state (201) thus attained will be strained. Presumably these factors diminished the β-elimination from C6 carbon and consequently contributing (200) as the minor product in the reaction.

Further investigations were centered on base promoted rearrangements of 4α,5α-epoxides. In this series, treatment of 4α,5α-epoxycholestan-3β-ol (167) with LDEA resulted only in 10% conversion to allylic alcohol Δ^5-cholesten-3β,4α-diol (202) and the unchanged starting material was re-isolated.

The removal of 6β-hydrogen by anti-elimination will account for the formation of Δ^5-cholesten-3β,4α-diol (202) (Fig. 96). Syn-elimination of 6α-hydrogen is probably inhibited because to bring 6α-hydrogen cis to the oxirane ring, will require deformation in ring B as discussed above.

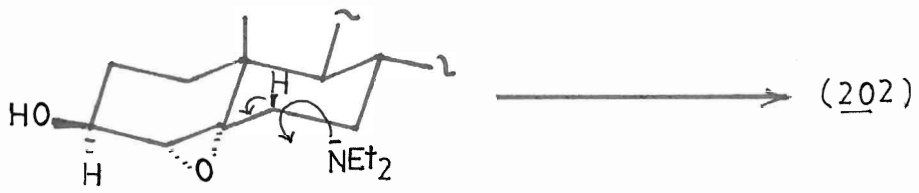
The corresponding reaction of 4α,5α-epoxycholestane (160) gave two products, Δ^3-cholesten-5α-ol (203)[103] (40%) and Δ^5-cholesten-4α-ol (204)[123] (15%).

Molecular model examination of epoxide (160) reveals the presence of two hydrogens, 3β and 6β, having trans disposition with respect to the oxirane ring. Abstraction of 3β-hydrogen in anti-elimination explains the formation of (203), while the removal of 6β-hydrogen accounts for the formation of the other isomeric allylic alcohol (204). The removal of 3β-hydrogen is more facile as it is less hindered compared to 6β-hydrogen and explains the formation of (203) as the major product (Figure 97).



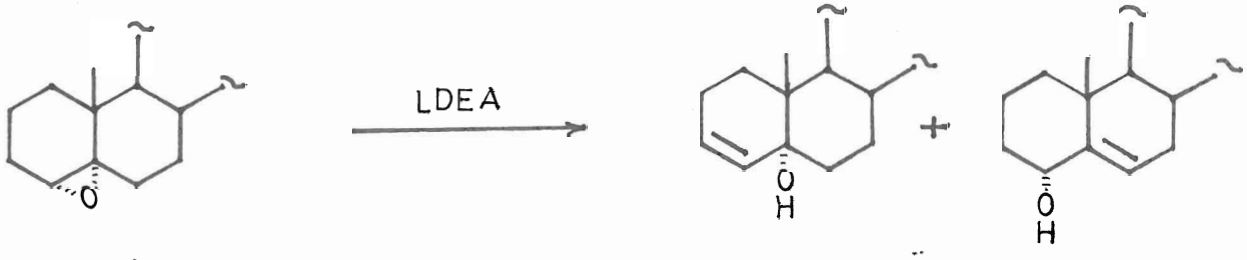
(167) (202)

Figure 96



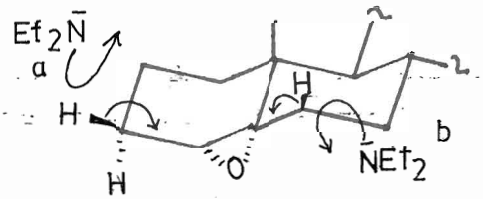
(202)

(167)



(160) (203) (204)

Figure 97



a

(203)

b

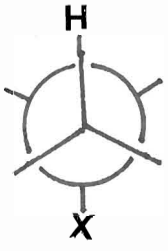
(204)

The product distribution in the LDEA-promoted allylic rearrangements of 4,5α-epoxides (160) and (167) shows that the β-elimination from C6 carbon is not very favourable. This is apparent from the formation of (202) and (204) only in minor amounts. This is perhaps because the 3β-hydrogen atom has an anti-periplanar arrangement[195] (Fig. 98) with respect to the oxirane ring. This arrangement is the most preferred arrangement for anti-elimination,[195] whereas, the 6β-hydrogens in these epoxides (160) and (167) show some deviation to somewhat anticlinal arrangement and therefore its elimination is not preferred. However, in case of epoxide (167) it was the only choice for elimination and so only 10% conversion was obtained.

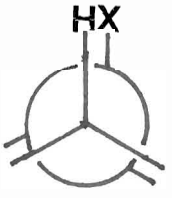
From the results obtained in the present investigation on base-promoted rearrangements of the steroidal epoxides, at least some conclusions can be drawn:

1. The inability of potassium t-butoxide to promote rearrangement in these epoxides may be due to its relatively weaker basic strength (compared with lithium amides) in abstracting hydrogen atoms β to the oxirane ring, or to its large steric size.
2. Failures observed using lithium diisopropyl amide (LDA) as the reagent are most probably due to its bulkiness which seems to prevent its approach close enough to abstract β hydrogens in these rigid molecules.
3. The presence of an ethylene ketal group at C3 carbon certainly has an activating effect on the C4 hydrogens to be removed in 5,6-epoxides.

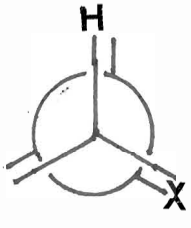
Figure 98



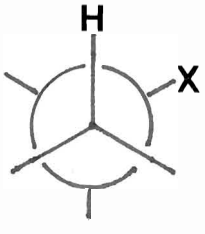
Anti periplanar



Syn periplanar



Anti clinal

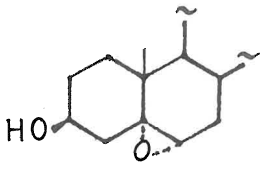
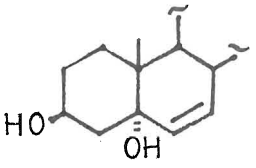
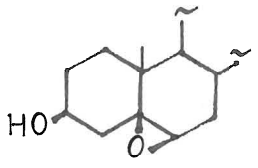
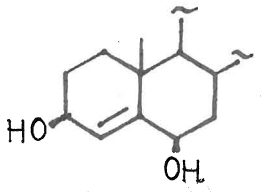
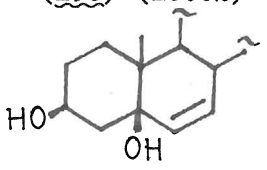
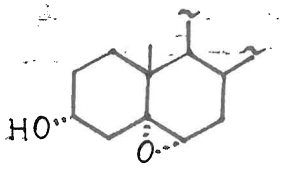
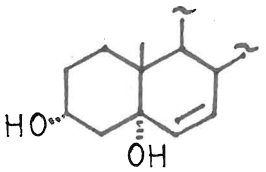


Syn clinal

4. The presence of 3β-hydroxyl group in 5α,6α-epoxide appears to have a long range deactivating effect on the C7 hydrogen abstraction by base. However, it does not effect the regiospecificity of the reaction. On the other hand, 3α-hydroxy group affects the regiospecificity by deactivating the C7 hydrogen to a greater extent, which may allow some β-elimination from the C4 carbon. Alternatively, this may be due to coordination of base to the 3α-hydroxyl group.
5. Rearrangements of 5β,6β-epoxides are generally complex, non-regiospecific, and slower relative to their corresponding α-isomers. However, 3β-hydroxyl group seems to have an activating effect on the C7 β-elimination, while the 3α-hydroxyl group deactivates both the C4 and C7 positions in these 5,6β-epoxides.
6. Rearrangements of 4,5-epoxides are also non-regiospecific and are highly effected by the presence of hydroxyl group at C3, by induced deactivation promoting β-elimination from C6 carbon.

It is apparent from the present study that much more effort involving labelling experiments is required in order to resolve the mechanistic details of base-promoted rearrangements of these steroidal epoxides.

Table 17. Reactions of Steroidal Epoxides with Strong Bases

Starting material	Reagent	Product(s)	Recovered starting material
 (2)	n-butyllithium	no reaction	≃95%
	potassium t-butoxide	no reaction	≃90%
	$LiNEt_2$	 (186) (56.4%)	
	$LiN(i\text{-}Pr)_2$	no reaction	≃90%
 (3)	n-butyllithium	no reaction	≃90%
	potassium t-butoxide	no reaction	≃90%
	$LiNEt_2$	 (190) (16.6%)  (189) (33.2%)	
	$LiN(i\text{-}Pr)_2$	no reaction	≃80%
 (125)	n-butyllithium	no reaction	≃90%
	potassium t-butoxide	no reaction	≃90%
	$LiNEt_2$	 (195) (18%)	

continued on next page

Table 17 (continued)

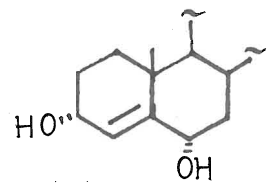
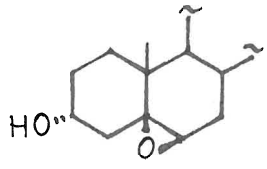
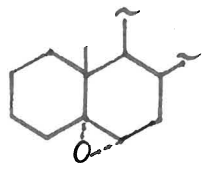
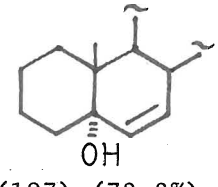
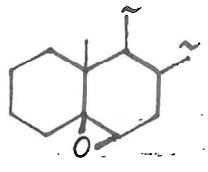
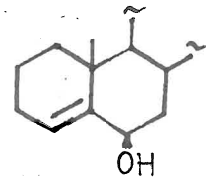
Starting material	Reagent	Product(s)	Recovered starting material
(125) continued	$LiNEt_2$ (continued)	 (194) (18%)	
	$LiN(i\text{-}Pr)_2$	no reaction	≃85%
 (126)	n-butyllithium	no reaction	≃95%
	potassium t-butoxide	no reaction	≃95%
	$LiNEt_2$	no reaction	≃75%
	$LiN(i\text{-}Pr)_2$	no reaction	≃85%
 (154)	n-butyllithium	no reaction	≃95%
	potassium t-butoxide	no reaction	≃90%
	$LiNEt_2$	 (187) (72.3%)	
	$LiN(i\text{-}Pr)_2$	no reaction	≃90%
 (155)	n-butyllithium	no reaction	≃90%
	potassium t-butoxide	no reaction	≃90%
	$LiNEt_2$	 (193) (5%)	≃90%
	$LiN(i\text{-}Pr)_2$	no reaction	≃90%

Table 17 (continued)

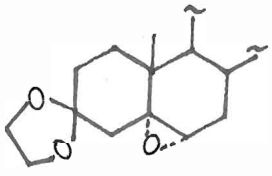
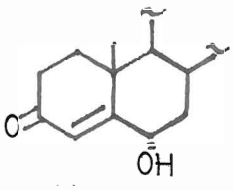
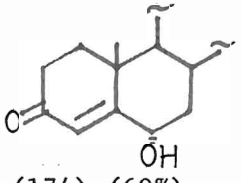
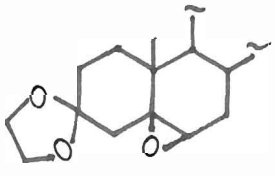
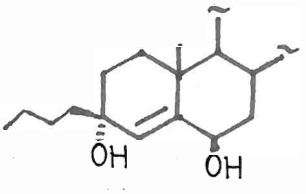
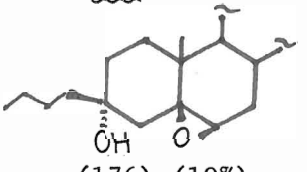
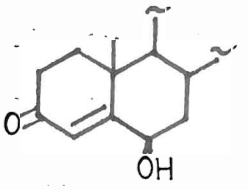
Starting material	Reagent	Product(s)	Recovered starting material
 (150)	n-butyllithium	 (174) (47%)	
	potassium t-butoxide	no reaction	≈90%
	$LiNEt_2$	 (174) (60%)	
	$LiN(i\text{-}Pr)_2$	no reaction	≈85%
 (151)	n-butyllithium	 (175) (75.5%)	--
	—	 (176) (10%)	
	potassium t-butoxide	no reaction	≈90%
	$LiNEt_2$	 (177) (29%)	
	$LiN(i\text{-}Pr)_2$	no reaction	≈80%

Table 17 (continued)

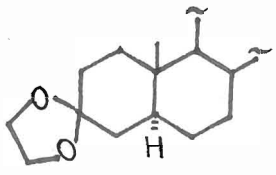
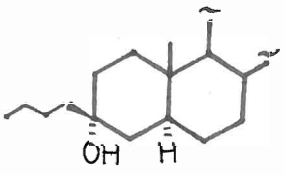
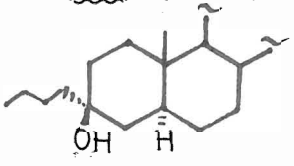
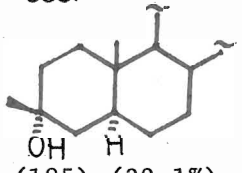
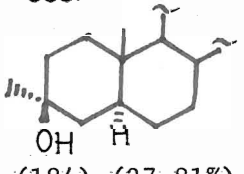
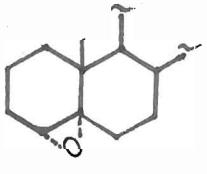
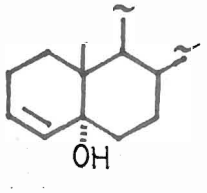
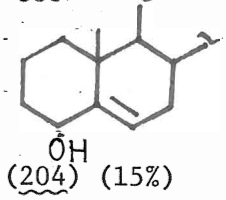
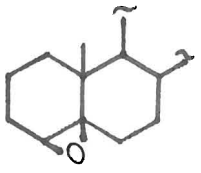
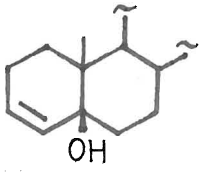
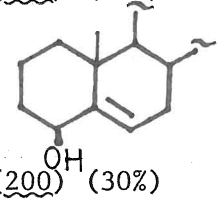
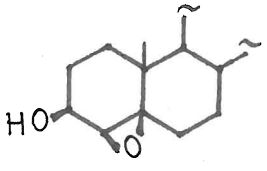
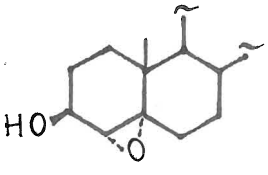
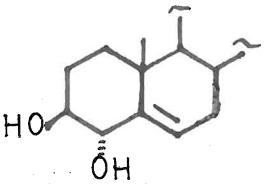
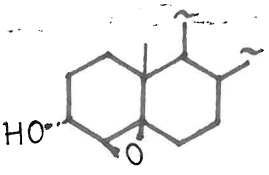
Starting material	Reagent	Product(s)	Recovered starting material
 (181)	n-butyllithium	 (182) (62.1%)	--
		 (183) (17.5%)	
	methyllithium	 (185) (32.1%)	40%
		 (184) (27.81%)	
 (160)	potassium t-butoxide $LiNEt_2$	no reaction	≃95%
		 (203) (40%)	
		 (204) (15%)	

Table 17 (continued)

Starting material	Reagent	Product(s)	Recovered starting material
 (161)	potassium t-butoxide	no reaction	≃90%
	$LiNEt_2$	 (199) (60%)  (200) (30%)	
 (164)	potassium t-butoxide	no reaction	≃90%
	$LiNEt_2$	no reaction	≃85%
 (167)	potassium t-butoxide	no reaction	≃90%
	$LiNEt_2$	 (202) (10%)	≃50%
 (169)	potassium t-butoxide	no reaction	≃95%
	$LiNEt_2$	no reaction	≃85%

SECTION-II

INTRODUCTION-II

INTRODUCTION-II

As a result of the pioneering work of several investigators over a period of 25 years, some 28 steroids have been isolated from the adrenal glands, and many from the urine, which appear to be related to adrenal metabolism, especially when associated with diseases of the adrenals. Only six of these steroids[197] from the adrenals possess biological activity, the others probably represent metabolic intermediates. All the three layers of adrenal cortex, namely, zona glomerulosa (outside), zona fasciculata and the zona reticularis (inside), are responsible for the formation of these corticoids, which in the form of a crude extract of cortex are known as cortin. The glomerulosa is believed to produce hormones responsible for electrolyte and water balance, the fasciculata, those affecting carbohydrate and protein metabolism.

When an animal is adrenalectomized, it dies soon afterward unless supported by injections of cortin. The important chemical findings after bilateral adrenalectomy are:[197]

1. Decreased Na^+, Cl^-, bicarbonate and glucose in the serum.
2. Decreased K^+ and NPN in the serum.
3. Decreased Na^+ in the muscle.
4. Increased K^+ and water in the muscle.
5. Decreased glycogen in the liver and muscle after fasting.
6. Increased excretion of Na^+ Cl^- and bicarbonate.
7. Decreased excretion of K^+.
8. Inability to excrete ingested water.

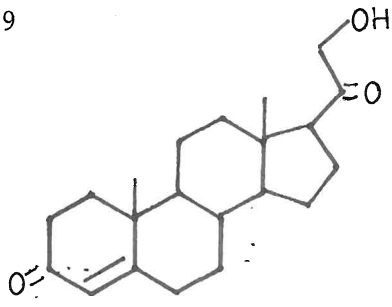
Figure 99 represents some important adrenal steroids. They are all hydroxylated and are characterized by the fact that in all the instances, C11 is either unsubstituted (11-deoxy series) or bears a ketonic or alcoholic functionality (11-oxygenated series). Several are active with respect to only one metabolic dysfunction produced by adrenalectomy. The 11-deoxycorticosterone (205) causes a retention of Na^+ and water, but is without effect in maintaining normal carbohydrate metabolism.[198] On the other hand, 17-hydroxycorticosterone (208) is active in its effect on carbohydrate metabolism but has no effect on sodium retention.[198]

The immediate precursor of these corticoids is the progesterone (213) which in turn itself is synthesized from cholesterol (1) *via* pregnenolone (214) in stereoidogenic tissues (Fig. 100).[199] To tell the whole story about the pathways of biosynthesis of these corticoids[200] from progesterone would require volumes.

In the intestine, these steroids are subjected to a variety of metabolic transformations before they are excreted. During these intestinal metabolic alterations, some of the steroids suffer dehydroxylations. The 21-dehydroxylation of corticosteroids by intestinal bacteria causes a shift from biliary to renal excretion.[201,202]

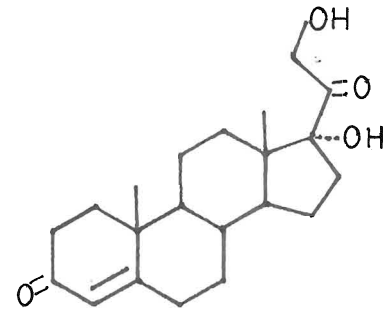
Bile acids are synthesized from cholesterol in the liver and their principal function is to facilitate the lipid absorption from the gut. They are returned with the lipids to the liver and undergo enterohepatic circulation. During these reabsorption cycles, they are used over and over again. In fact, less than 5% of bile acids are lost in the faeces per circulation.

Figure 99



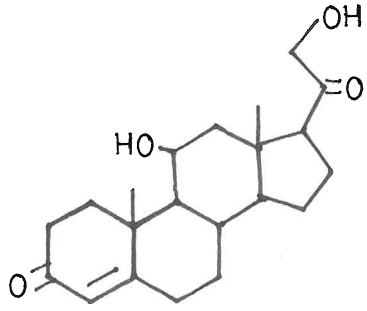
(205)

11-Deoxycorticosterone



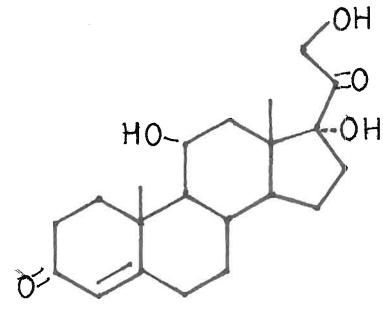
(206)

Cortexolone



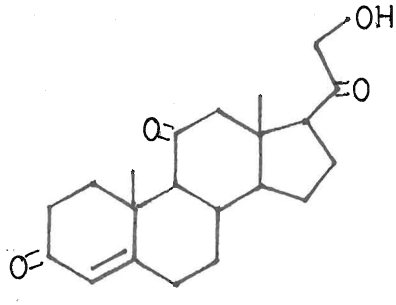
(207)

Corticosterone



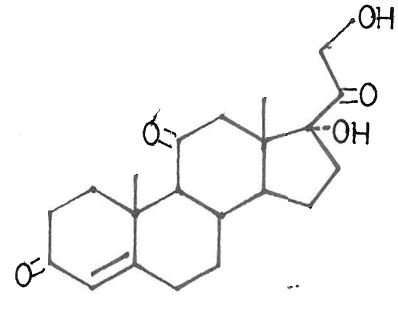
(208)

Cortisol



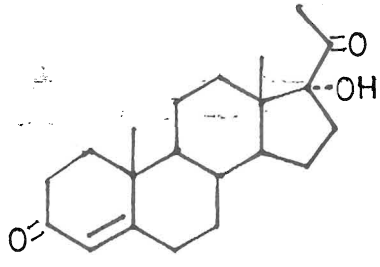
(209)

11-Dehydrocorticosterone



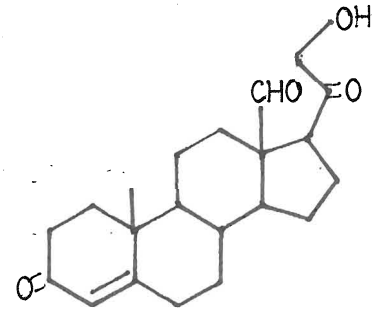
(210)

Cortisone



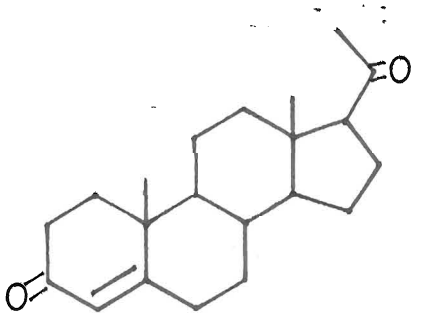
(211)

17-Hydroxyprogesterone

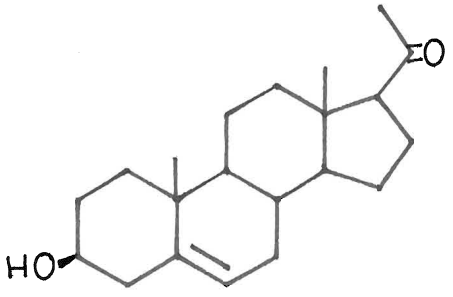


(212)

Aldosterone

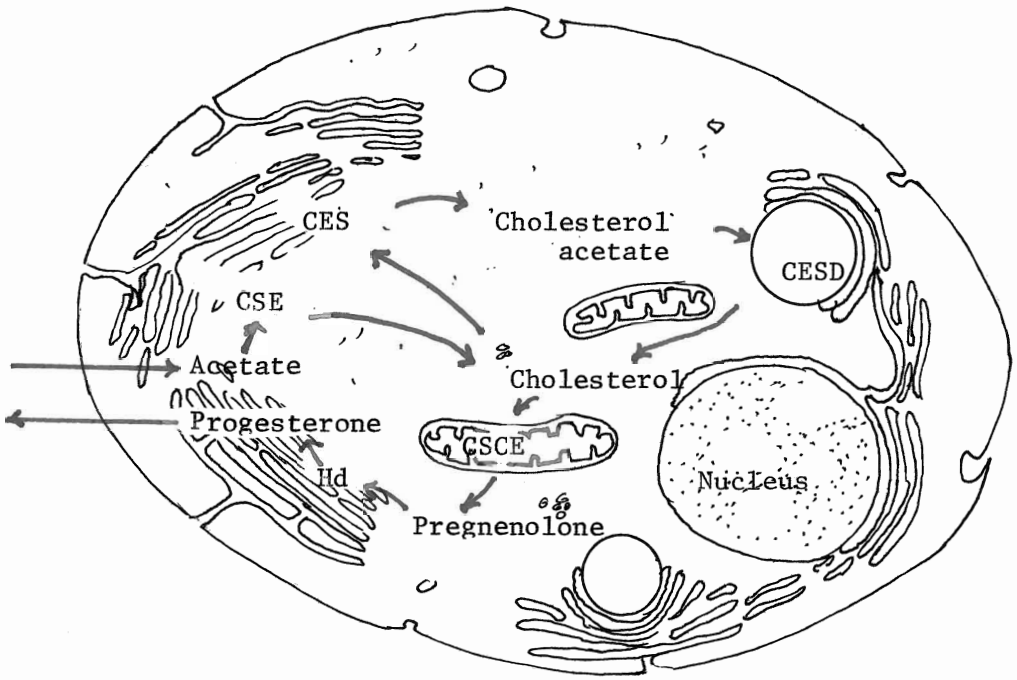


(213)



(214)

Figure 100

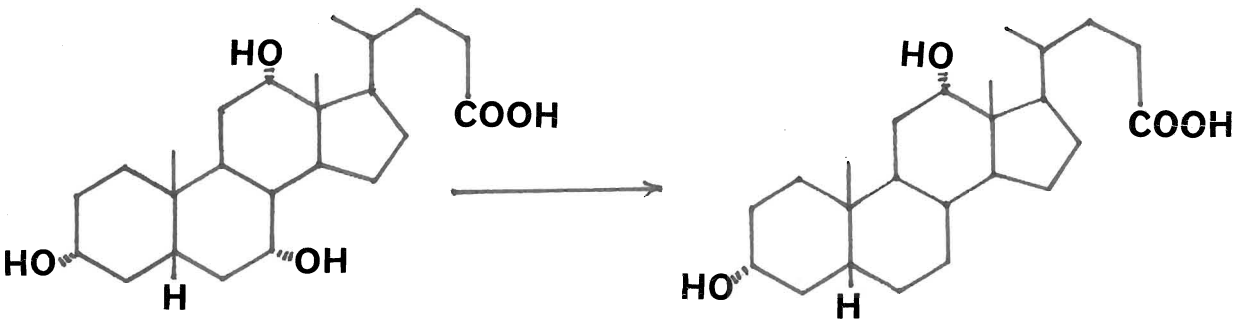


Biosynthesis of Progesterone in stereoidogenic tissues

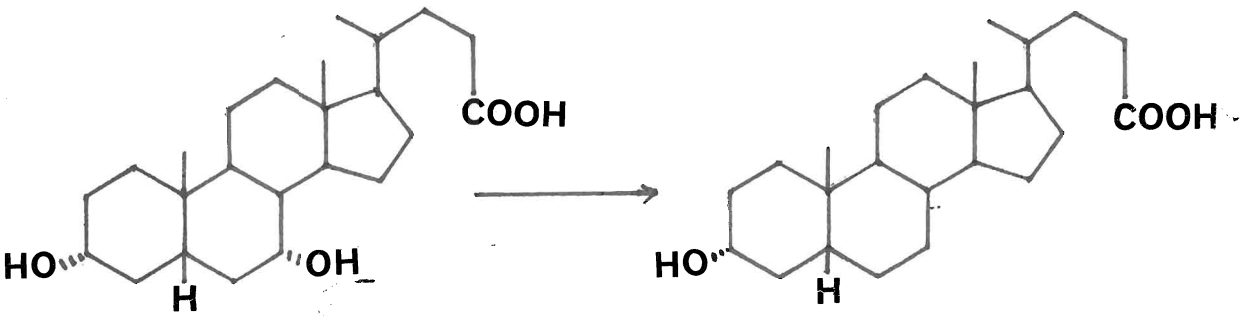
CSE,cholesterol synthetase enzyme; CES,cholesterol ester synthetase CESD,cholesterol ester storage droplet; CSCE,cholesterol side chain cleave enzyme; Hd,3$\overline{\beta}$-hydrogenase

The composition of bile acids in biliary excretions of man is determined by both liver and biotransforming enzymes and intestinal bacterial transformation reactions.[201] The two most important biotransformations carried out by intestinal bacteria are deconjugation of the conjugated bile acids yielding free bile acids and the dehydroxylation for example, the 7α-dehydroxylation of cholic acid (215) and chenodeoxycholic acid (217) forming deoxycholic acid (216) and lithocholic acid (218) respectively.[203]

Lindstedt et al.[204,205] demonstrated that deoxycholic acid (216) is not a primary bile acid, i.e., derived from cholesterol in the liver, but is formed by the bacterial dehydroxylation of cholic acid (215) by the intestingal flora. Other studies[206-208] have shown that not only deoxycholic acid (216) but a great variety of bile acids are formed by bacterial action on the hydroxy substituents of primary bile acids. The secondary bile acids thus produced are more "toxic" than their corresponding primary bile acids and have been implicated in the etiology of a number of diseases of the gastrointestinal system.[209] For example, lithocholic acid (218) has been reported to be hepatotoxic, to cause hyperplasia[210] and liver tumours,[209] to enhance mutagenicity[211] and tumorgenicity[212] of known chemical carcinogens, and to cause DNA strand break in mouse lymphoblastoma cells.[213] Moreover, Low-Beer and Nutter[214] have presented evidence which suggests that deoxycholic acid (216) may be important in increasing the cholesterol saturation of bile acids and potentially increasing the risk of cholesterol gall stone formation.



(215) (216)



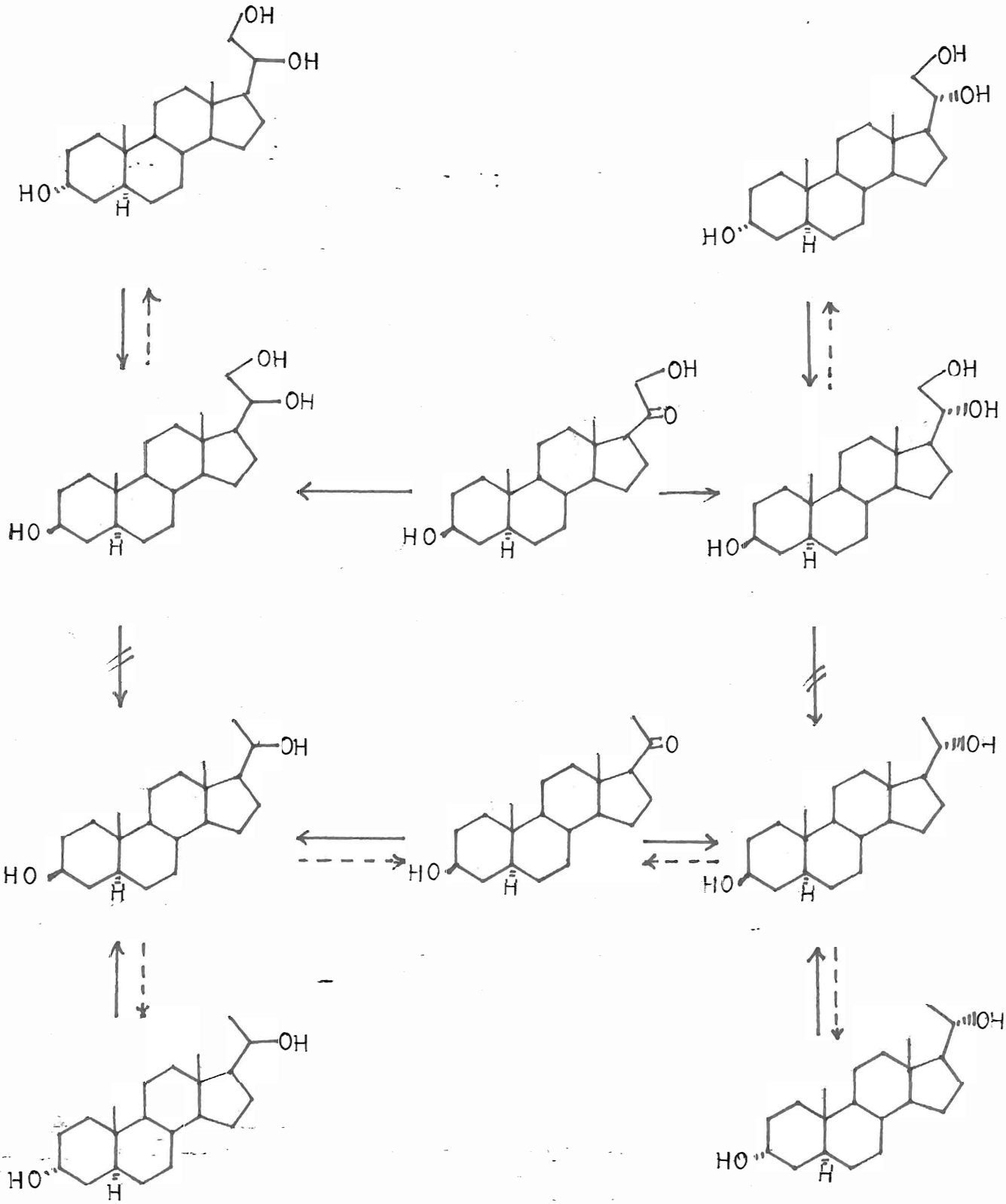
(217) (218)

Some of these bile acids are absorbed *via* the portal system and excreted in the bile.[208-210] During their passage through the liver, secondary bile acids may be further metabolized either to primary bile acids from which they originated, or to still other cholanic acids.[215] The important concept thus emerged that the bile acids found in the bile consist of a mixture of primary and secondary bile acids that reflect a complex series of hepatic and bacterial transformations.

More than a decade ago, Gustafsson[216] and Gustafsson *et al.*[217] provided evidence that the intestinal flora of rats synthesized steroid metabolising enzymes which were not present in mammalian tissues. The predominant steroids in faeces from germ-free rats carry a 21-hydroxyl group, while no 21-hydroxylated steroid was identified in the faeces from conventional rats[216,218] (Fig. 101). These findings indicated microbial participation in a 21-dehydroxylation by intestinal flora. Besides undergoing 21-dehydroxylations, the 21-hydroxy-2-oxo-steroid was reduced and once the 20,21-diol structure has been formed as the result of the metabolism in microorganism or in the host tissues, microbial dehydroxylation can no longer take place.[219]

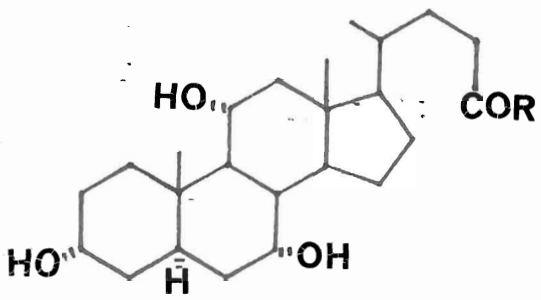
It has been shown that the administered cholesterol is converted in the liver to the primary bile acid, allocholic acid (220), which is excreted in bile as glycoallocholate (221). In the large intestine, bacterial 7α-dehydroxylation and hydrolysis of the peptide bond produces allodeoxycholate (222), which is absorbed, conjugated with glycine (219) and excreted in the bile as glycoallodeoxycholate (223) which in turn is precipitated by calcium ions in the gall bladder to produce cholelithiasis[220] (Fig. 102).

Figure 101



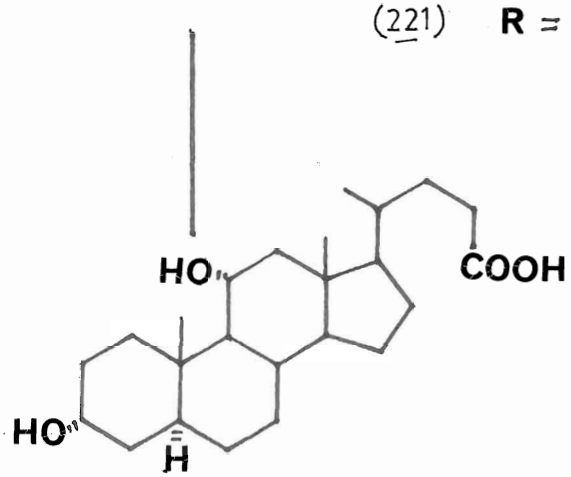
Conversion of 3β20-dihydroxy-5α-pregnan-20-one by micro organisms from rat caecal flora.

Figure 102



(220) R = OH

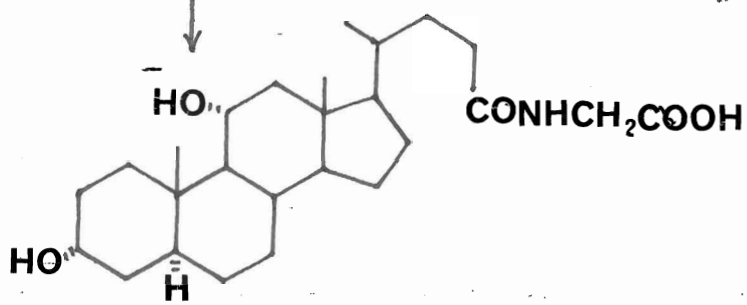
(221) R = $NHCH_2COOH$



(222)

$\overset{+}{N}H_3CH_2COO^-$

(219)

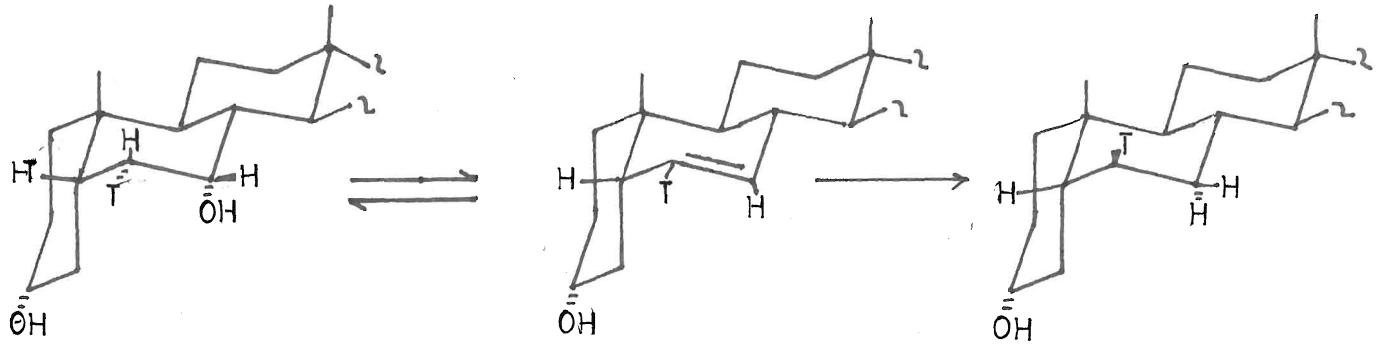


(223)

Absence of dehydroxylation in the germ-free animals or its suppression by treatment with antibiotics should cause a decrease of allodeoxycholate formation leading to lower concentrations of allodeoxycholate in bile and subsequent prevention of cholelithiasis. In 1968, Mosback et al.[221] demonstrated that neomycin prevented cholelithiasis. The activity of faecal 7α-dehydroxylase, which converts primary bile acids to secondary bile acids was found to be higher in patients with colon cancer than in controls.

7α-Dehydroxylation activity has been detected in members of several genera of anaerobic intestinal bacteria including Bacteroides,[222] Clostridium[223] and Eubacterium.[224] 7α-Dehydroxylation of bile acids is unique reaction in steroid biochemistry involving both a putative dehydration and a reductive step. The proposed reaction mechanism elucidated by Samuelsson[225] is shown in Figure 103. The initial step in this biotransformation is believed to proceed via a diaxial trans elimination of elements of water resulting in the release of 7α-hydroxy group and the 6β-hydrogen. The resulting Δ^6-intermediate is subsequently reduced by trans-hydrogenation at the 6α- and 7β-positions to yield deoxycholic acid. Recently, White et al.[226] showed that the reduction of Δ^6-intermediate required cell extracts prepared from cultures of Eubacterium species, V.P.I. 12708 grown in the presence of cholic acid and NAD^+. The cofactor requirements for 7α-dehydroxylation suggested that the bacterium may use this reaction as a mechanism to dispose of electrons generated by fermentative metabolism. It is also possible that secondary bile acids may be sufficiently toxic to other intestinal bacteria so as to provide a

Figure 103



selective advantage for 7α-dehydroxylating bacteria. This mechanism still needs more investigation.

In 1962, Calvin and Liberman[227] demonstrated that 16α-hydroxy-progesterone (224) administered intravenously to a human, was excreted in the urine as 3α-hydroxy-5β,17α-pregnan-20-one (225). Thus prior to excretion, the molecule had undergone ring reduction, dehydroxylation at C16 and change in the configuration of the side chain from β to α.

In humans, 16α-hydroxyprogesterone (224) is reduced in the liver to 16α-hydroxypregnanolone (226).[228-231] In rats,[232-234] the steroid is reduced to 3α,16α-dihydroxy-5α-pregnan-20-one (227). In both the species,[230,231] 16α-hydroxy steroids are excreted in the bile, undergo enterohepatic circulation, and therefore become exposed to the intestinal flora.

In conventional rats, the biliary 3α,16α-dihydroxy-5α-pregnan-20-one (227) is dehydroxylated to 3α-hydroxy-5α,17α-pregnan-20-one (228) which is excreted in the faeces.[236] On the other hand, germ-free rats do not alter 16α-hydroxy steroid.[236] These findings suggested the presence of 16α-dehydroxylase in faecal flora, which was further supported by incubations of 3β,16α-dihydroxy-5α-pregnan-20-one (229) with faecal bacteria[236] and intestinal flora of man.[237] In both the cases, the major metabolite was 3β-hydroxy-5α,17α-pregnan-20-one (230). This follows that the intestinal flora are responsible for the molecular alterations and that 16α-hydroxyprogesterone (224) is the precursor for urinary 17α-pregnenolone (Fig. 104). The faecal flora reduced the 3-keto group, removed the hydroxyl group at C16 carbon, and isomerized the C17 side chain from the

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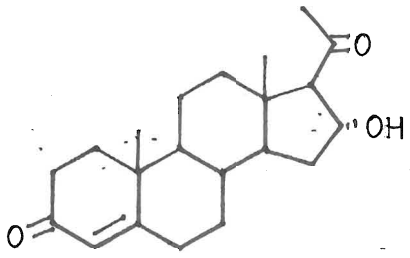
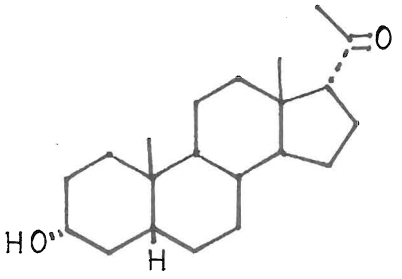
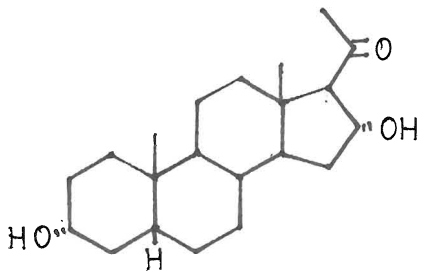


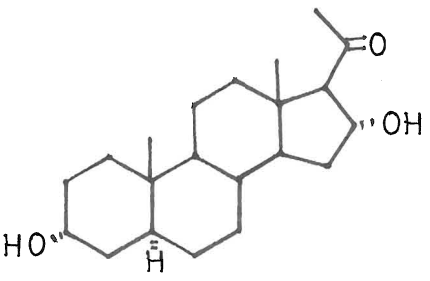
(224)



(225)



(226)



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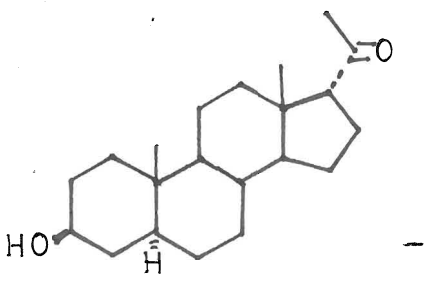
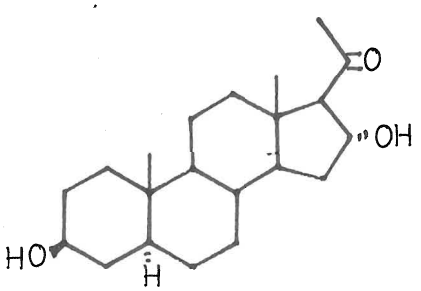
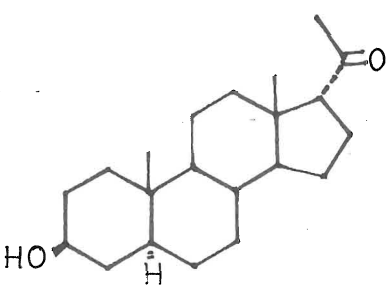


(228)

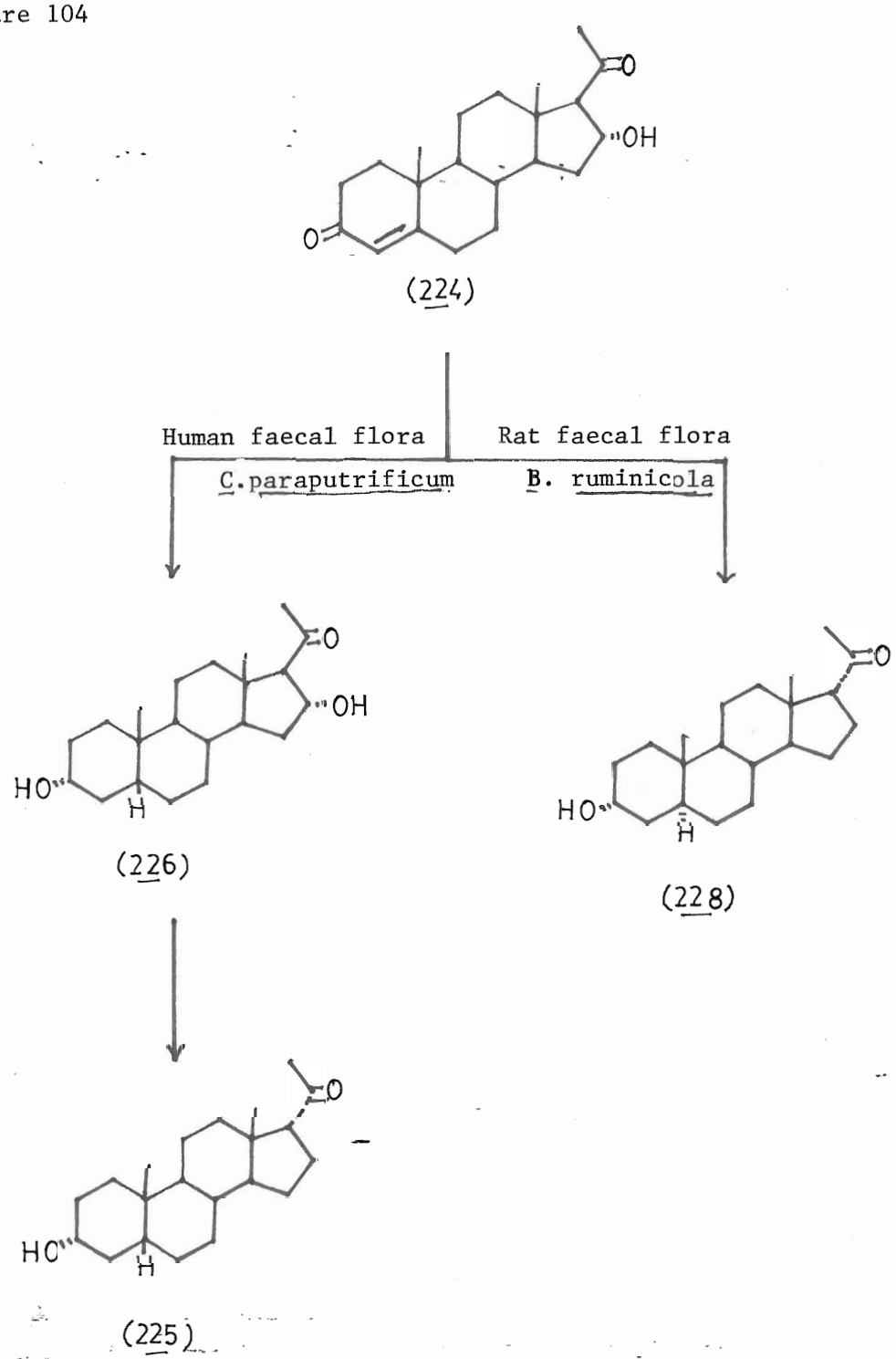


(229)



(230)

Figure 104


O
"OH
O
(224)
Human faecal flora
Rat faecal flora
C.paraputrificum
B. ruminicola
O
"OH
HO"
H
(226)
O
HO"
H
(228)
O
HO"
H
(225)

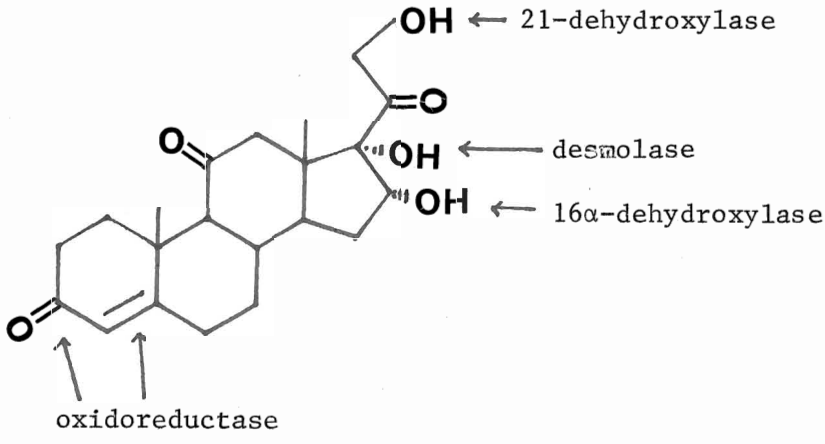
β- to α- configuration. The ring A is reduced to 5β stereochemistry in humans, and 5α in rats.[238]

Calvin and Liberman[227] have suggested that the conversion of 16α-hydroxyprogesterone (224) to 17α-pregnanolone (225) takes place via the intermediate C21-Δ^{16}-steroid, which is then metabolized in vivo to 17α-pregnanolone (225). Bokkenhauser et al.[238] could not detect this C21-Δ^{16}-steroid in the cultures. Presumably, if present, it was rapidly reduced or alternatively, it never detached from the enzyme surface. The mechanism of 16α-dehydroxylation is yet not clear and requires more investigation.

The importance of bacterial metabolism of biliary steroids has become more evident in recent years.[231,239] In many mammalian species, including man, the steroid metabolites are absorbed, conjugated and eventually excreted in the urine, while in some mammals most of the biliary steroids are ultimately excreted in faeces. In both types of mammals, structures of urinary and biliary steroids differ markedly. The principal types of bacterial transformations of a steroid[240] molecule are shown in Figure 105. For unknown reasons, cortisol (208) does not undergo biliary excretion in man, although it does in cats[241] and rats.[242]

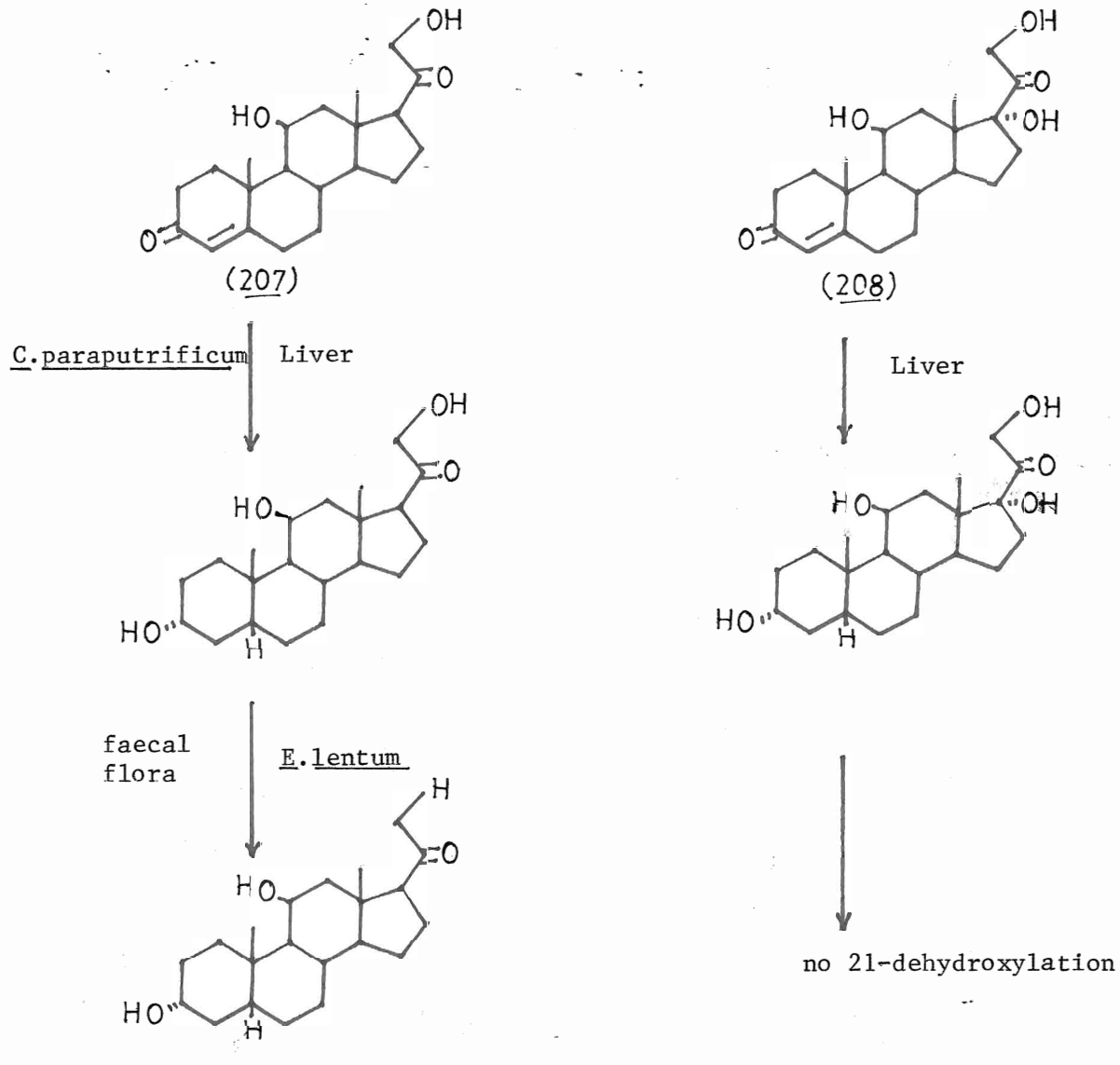
21-Dehydroxylation of corticosteroids, in rats[239,244-246] as well as in humans,[218,219,243] appears to be restricted to those steroid hormones which undergo enterohepatic circulation. The Δ^4-3-oxo-structure of many corticoids (Fig. 106) gets saturated[230] during their 21-dehydroxylations. The modified steroids are excreted into the bile and like bile acids, undergo enterohepatic circulation. The reason for this phenomenon is

Figure 105



Structures of corticosteroids susceptible to bacterial transformations

Figure 106


OH
O
HO
O
(207)
C.paraputrificum
Liver
OH
O
HO
HO
H
faecal
flora
E.lentum
H
O
HO
HO
H
OH
O
HO
"OH
O
(208)
Liver
OH
O
HO
"OH
HO
H
no 21-dehydroxylation

unknown. Ring A-saturated and conjugated metabolites derived from 11-deoxycorticosterone (DOC) (205), 11-dehydrocorticosterone (209) and corticosterone (207) by hepatic metabolism are excreted into the bile[239,247,248] (Fig. 107). Deoxycorticosterone (DOC) (205) has been shown to be a precursor of the urinary metabolite, 3α,20-dihydroxy-5β-pregnane glucuronide,[245] Dehydrocorticosterone (209) and corticosterone (207) have been demonstrated to be precursors of 3α,20α-dihydroxy-5β-pregnan-11-one-glucuronide excreted in the urine[201,202] (Fig. 107). Aldosterone[249] (231) is metabolized to 21-deoxytetrahydroaldosterone (232) and a 21-deoxy-bicyclic acetate (233) (Fig. 108). The metabolites of cortisol (208) are not significantly excreted in bile.[247,250] Fukushima and Gallagher[248] have demonstrated that cortisol does not undergo 21-dehydroxylation.

The involvement of intestinal bacteria in 21-dehydroxylation reactions was first demonstrated by Gustafsson[216] and Gustafsson and Sjovall.[218] Eriksson and Gustafsson[237] studied biotransformation reactions catalyzed by faecal contents recovered from the ileostoma of a calectomized patient and observed the 21-dehydroxylation of 3β,21-dihydroxy-5α-pregnan-20-one (234) yielding 3β-hydroxy-5α,17β-pregnan-20-one (235) (Fig. 109). Bokkenheuser et al.[251,252] described 21-dehydroxylation of deoxycorticosterone (205) in cultures of mixed faecal flora of normal individuals. In more recent studies, Bokkenheuser et al.[253] succeeded in isolating from human faeces an obligate anaerobe capable of 21-dehydroxylating corticosteroids with an α-ketal side chain.[254] The precise identity of the bacterium has not yet been established, although it has been classified as an Eubacterium lentum like organism,[259] and shares many of the properties of the latter.

Figure 107

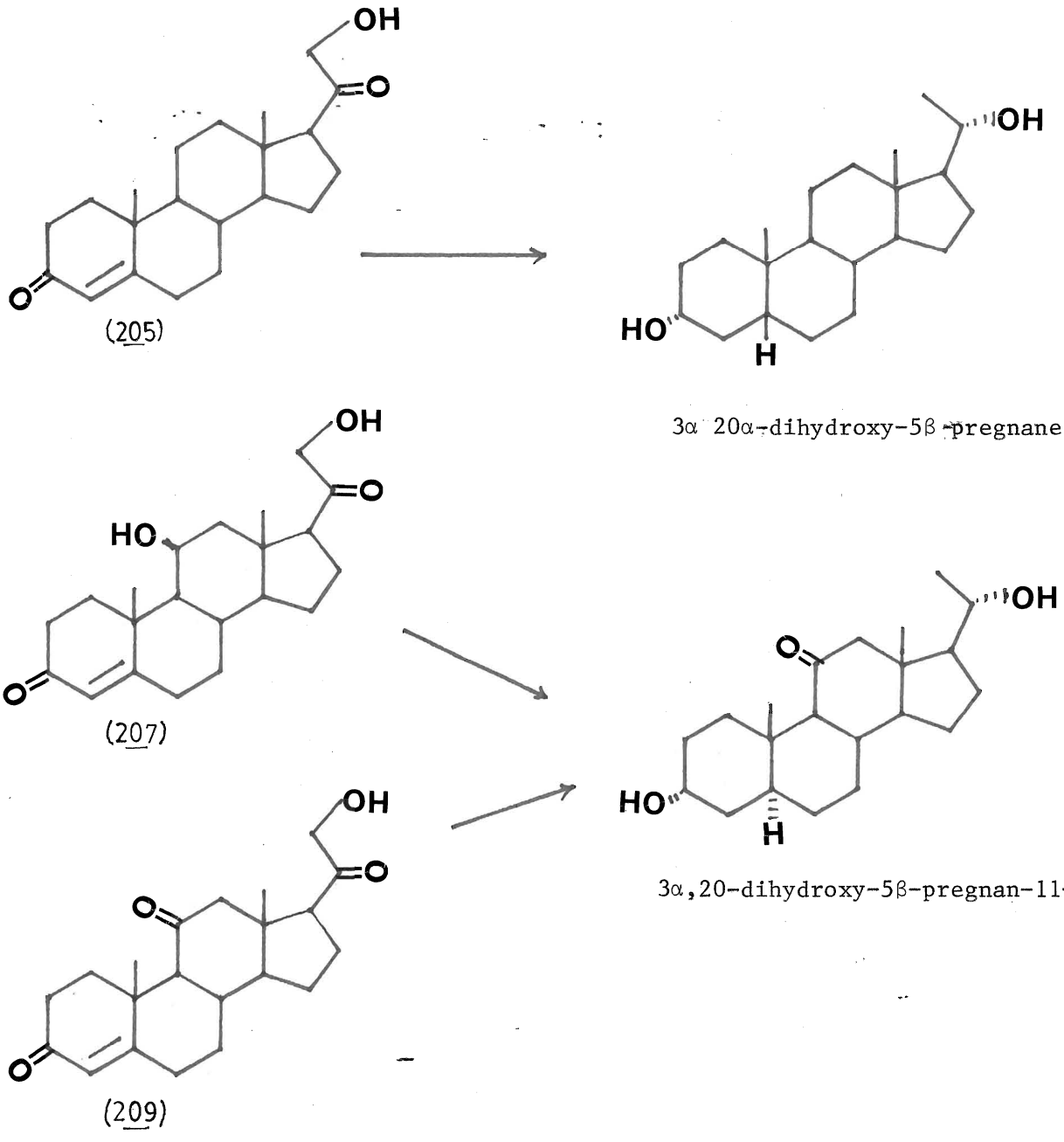
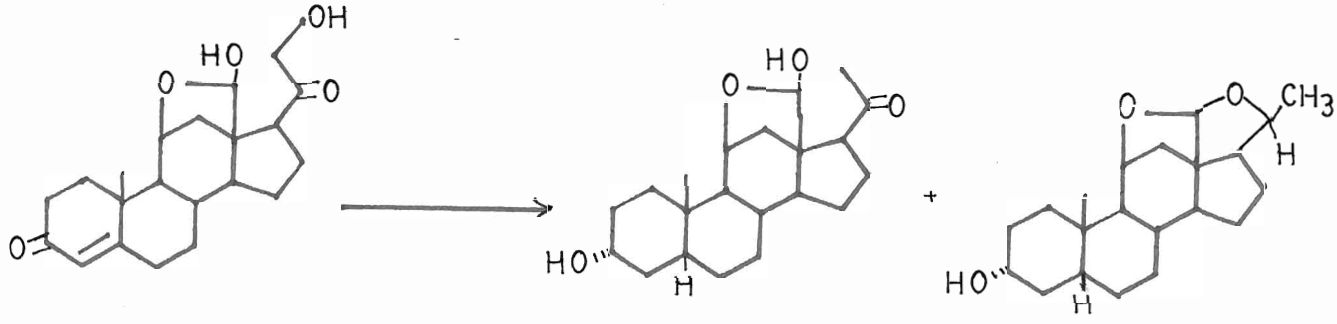

OH
O
O
(205)
OH
HO
H
3α 20α-dihydroxy-5β-pregnane
OH
O
HO
O
(207)
OH
O
HO
H
3α,20-dihydroxy-5β-pregnan-11-one
OH
O
O
O
(209)

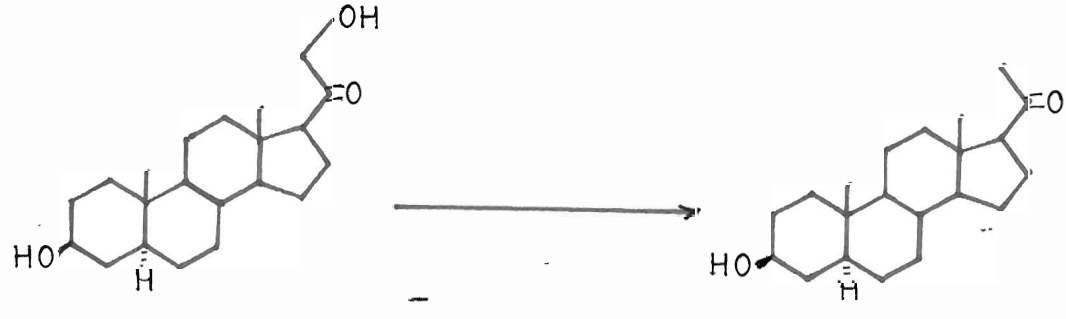
Figure 108



(231) (232) (233)

3α-hydroxy-5β-pregnan-
(11β-18s)(18S-20α)-dioxide

Figure 109



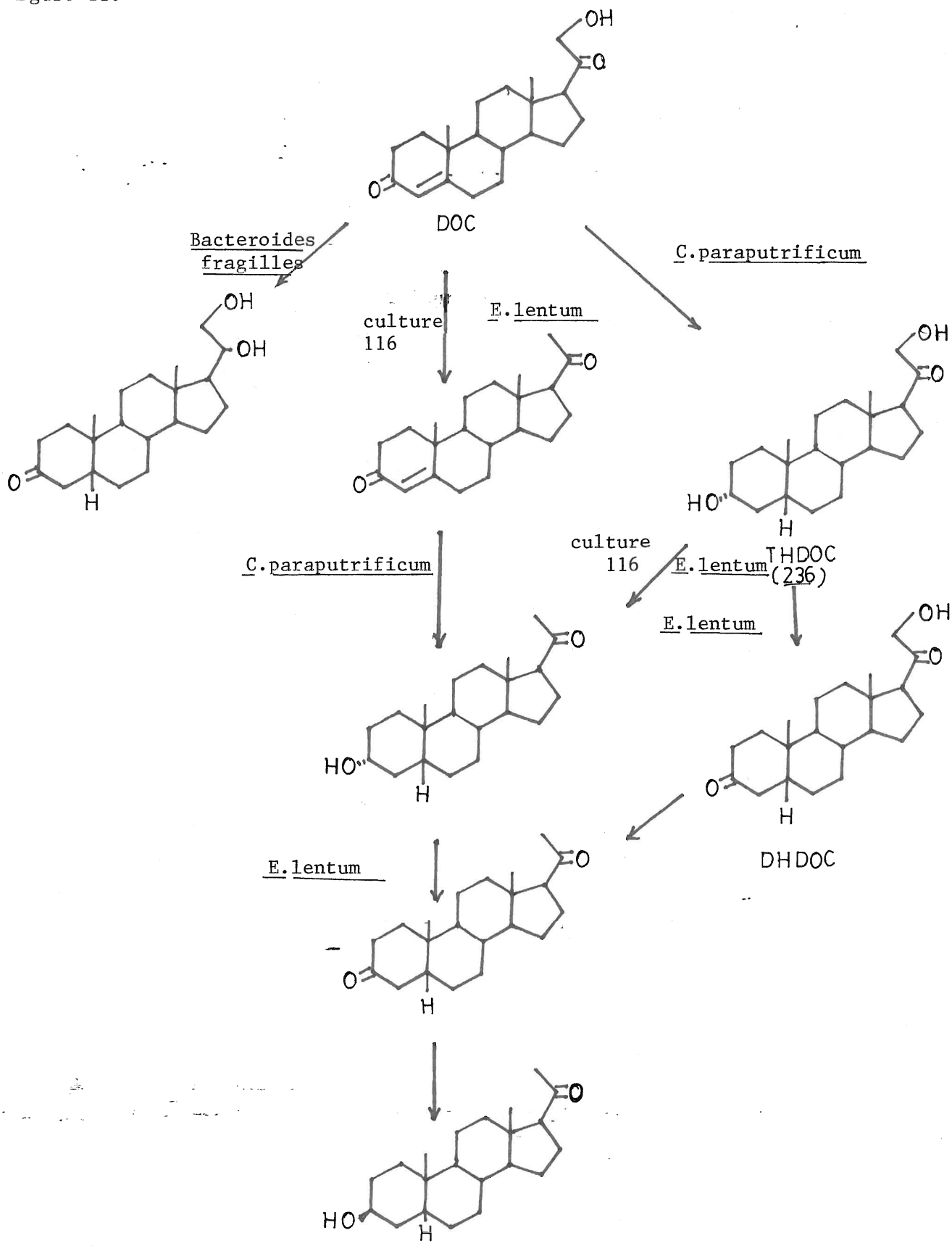
(234) (235)

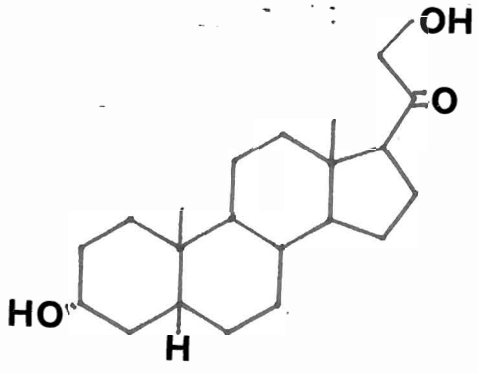
Recently[255] an oxygen sensitive corticosteroid 21-dehydroxylase has been characterized in the cell extracts of Eubacterium lentum (V.P.I. 11122, formerly culture 116). The enzyme is highly specific for corticoids containing α-ketal structure and requires $FMNH_2$ or reduced benzyl viologen for activity. The enzyme can use deoxycorticosterone (205), deoxycortisol (206), dehydrocorticosterone (207) and corticosterone as substrates. Substrate saturation kinetics using [^{3}H]-corticosterone has yielded an apparent K_m of 7.35 μM and a V_{max} of 15.4 nmol (11β-[^{3}H]-hydroxyprogesterone) formed (per hour.mg protein^{-1}). The enzyme has an apparent molecular weight of 582,000.

Bacterially mediated metabolic pathways of deoxycorticosterone and allied steroids metabolism are shown in Figure 110. Deoxycorticosterone (205) is rapidly reduced to tetrahydrodeoxycorticosterone (THDOC) by C. paraputrificum and to a much lesser degree by other intestinal bacteria. At Eh -250 to -300 mV, THDOC (236) is 21-dehydroxylated to pregnanolone (237) by culture 116 and the obligate anaerobe related to Eubacterium lentum (Fig. 110). This is the preferred pathway[252] in cultures of mixed faecal flora.

In an alternate pathway, culture 116 21-dehydroxylates deoxycorticosterone (205) to progesterone (213), while the reduction of ring A to pregnanolone (214) is accomplished by C. paraputrificum. The reduction of ring A is not prerequisite for 21-dehydroxylation, nor is a 21-hydroxyl group necessary for ring A reduction. On the other hand, the presence of 20-hydroxyl group in 20,21-diol metabolites protects both the 3-keto and 21-hydroxyl against bacterial enzymes.[252]

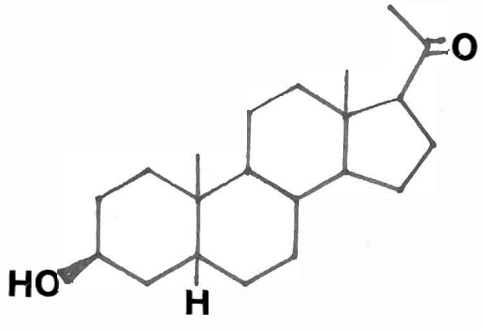
Figure 110


OH
O
O
DOC
Bacteroides fragilles
C.paraputrificum
culture 116
E.lentum
OH
OH
O
H
O
O
HO
H
OH
O
THDOC (236)
culture 116
E.lentum
C.paraputrificum
E.lentum
O
HO
H
OH
O
O
H
DHDOC
E.lentum
O
O
H
HO
H
O
(237)



Tetrahydrodeoxycorticosterone

(236)



5β-Pregnan-3β-ol-20-one

(237)

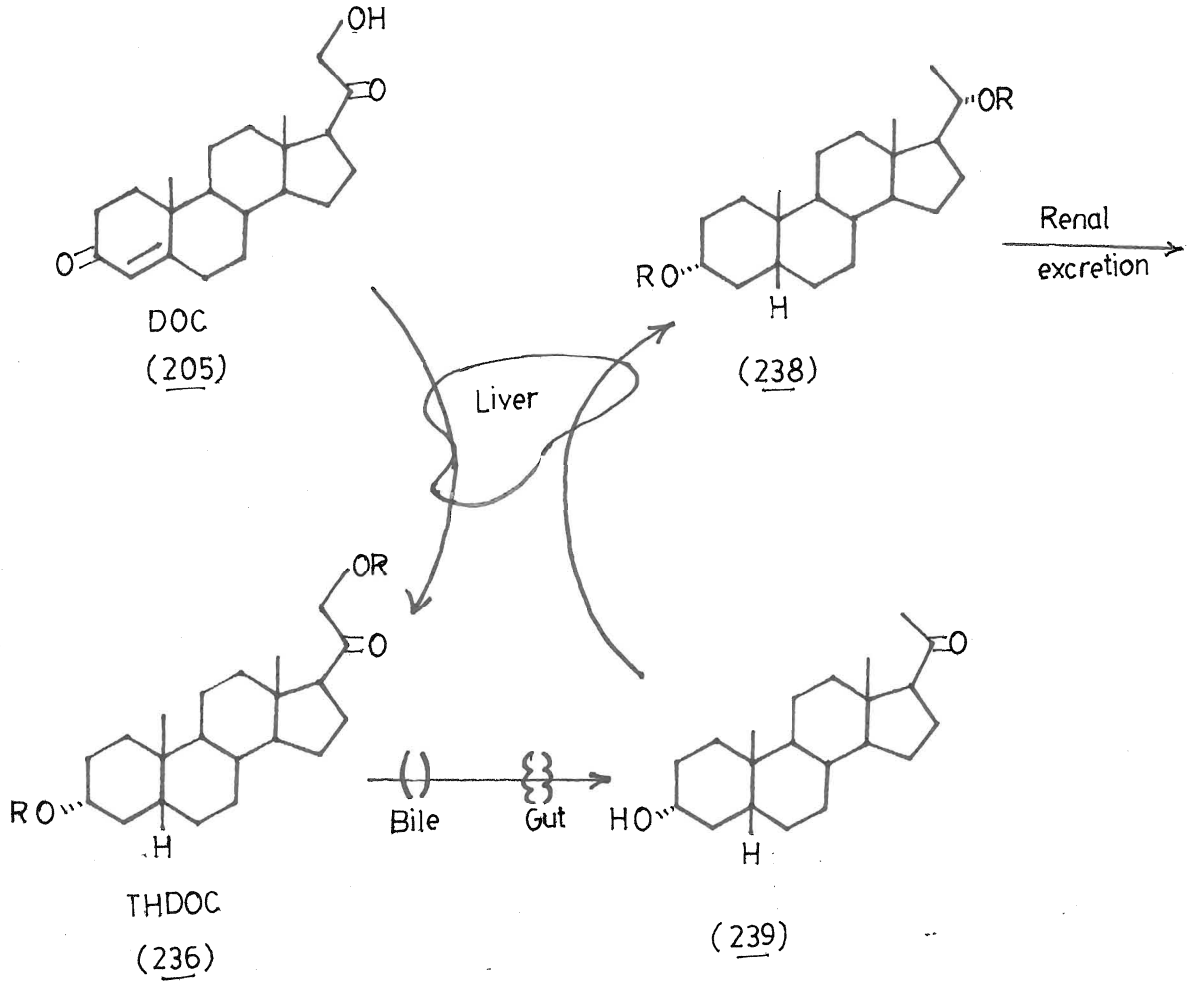
Proposed metabolism of DOC in vivo[256]

In humans,[245] urinary 3α,20α-dihydroxy-5β-pregnane (238) is a 21-dehydroxylated derivative of DOC for the formation of which in vivo, the metabolic pathway is depicted in Figure 111. Deoxycorticosterone (205), is reduced to THDOC (236) in the liver, conjugated, excreted in the bile, transported into the intestine, probably deconjugated and dehydroxylated by culture 116, E. Lentum or an allied bacteria to 5β-pregnanolone (239). Although the fate of 5β-pregnanolone (239) is unclear, it has been suggested[252] that it is absorbed, reduced in the liver to 3α,20α-dihydroxy-5β-pregnane conjugated with glucuronic acid (240) and returned to the blood for renal excretion.[256]

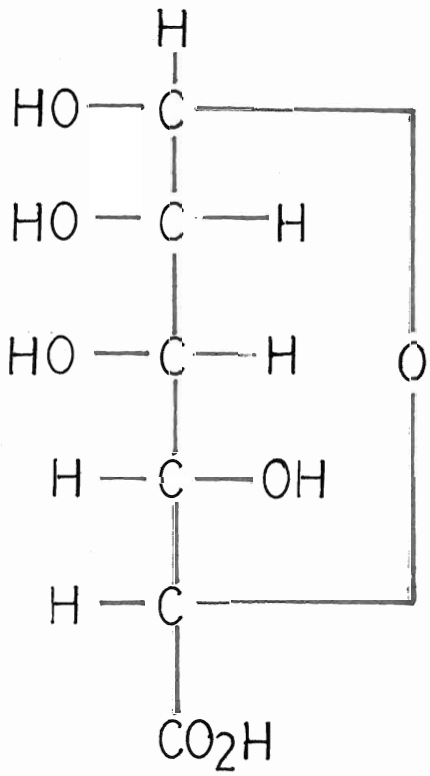
The optimal conditions under which cells of E. lentum carry out 21-dehydroxylation is not yet clear. The rate of conversion of deoxycorticosterone (205) to progesterone (213) was found to be greatly stimulated when Escherichia coli was grown as a mixed culture[255] with E. lentum with subsequent lowering of the medium Eh from -80 mV to -280 mV. It is believed that the role of E. coli in the transformation of steroids, in vitro, is to provide a suitable redox environment not only for the multiplication of the bacteria, but also for the function of the steroid metabolizing enzymes.[255]

Recently, Scott and Phillip[255] observed a sevenfold increase in the rate of conversion of deoxycorticosterone (205) to progesterone (213) in whole cell suspension of E. lentum when H_2 was sparged through the reaction mixture as compared to argon gas. They suggested that H_2 (via hydrogenase) may provide reducing equivalents for 21-dehydroxylation of

Figure 111



Proposed in vivo metabolic pathway of deoxycorticosterone undergoing enterohepatic circulation R= glucuronic acid



(D)-Glucuronic acid

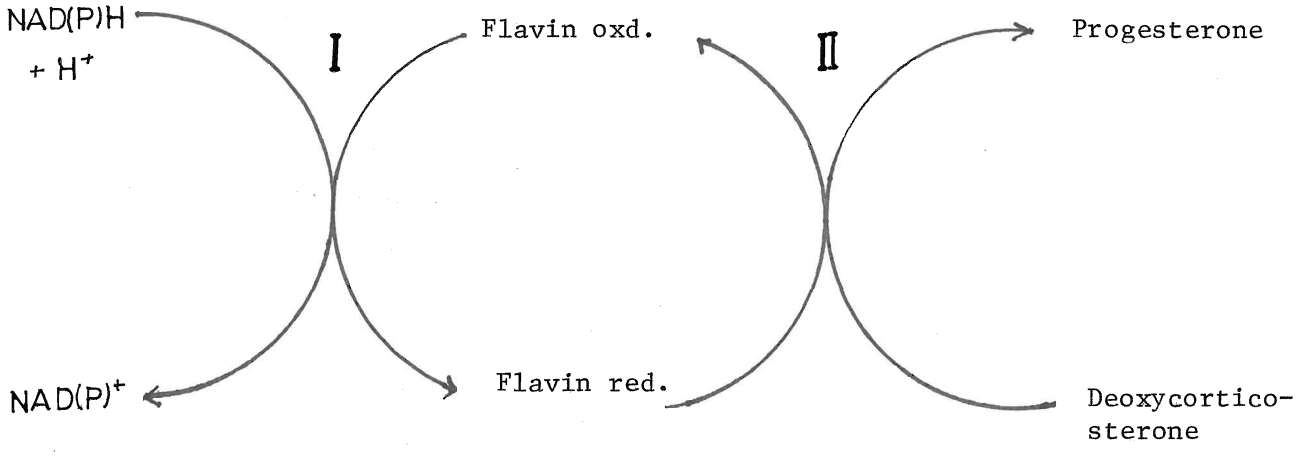
(240)

deoxycorticosterone (205). Since E. coli is capable of H_2 formation via formic hydrogenlyase, this may account for the stimulation of 21-dehydroxylation of deoxycorticosterone (205) in the mixed culture.

The 21-dehydroxylase activity has also been reported[255] to be inhibited by both water soluble metal ion chelating agents, NaCN, NaN_3 and EDTA, and the lipophilic metal ion chelating agnets, merasyl, o-phenathroline, 8-hydroxyquinoline, and α,α'-dipyridyl.

Bokkenheuser et al.[257] in 1979 and Scott and Phillip[255] in 1980 have reported that anaerobically dialyzed cell extracts of E. lentum had an absolute requirement for both reduced pyridine nucleotide (either NADH or NADPH) and an oxidized flavin (FAD or RMN) for the 21-dehydroxylation of deoxycorticosterone (205). However, photochemically reduced flavin FMN ($FMNH_2$) could replace the requirement for a reduced pyridine nucleotide (NAD(P)H) and oxidized flavin.[255] The 21-dehydroxylase was found to be active from pH 5.4 to 8.6 with an apparent optimum between pH 6.4 and 6.8 using a mixture of NADH plus FMN as coenzymes. The electron model for the 21-dehydroxylation of deoxycorticosterone (205) using photochemically reduced FMN($FMNH_2$) is represented in scheme[255] shown in Figure 112. This pathway included a NAD(P)H:flavin oxidoreductase (I) for generating reduced flavins which in turn are re-oxidized by 21-dehydroxylase (II) during the biotransformation of deoxycorticosterone (205) to progesterone (213). Moreover, it has been demonstrated[255] that NADH:FMN oxido-reductase and 21-dehydroxylase are not physically associated. 17α-Hydroxyprogesterone (211) has been found[255] to inhibit the 21-dehydroxylation of [^{3}H]-deoxycorticosterone in mixed substrate culture. However, Winter et al.[254]

Figure 112



21-Dehydroxylation of deoxycorticosterone in E.lentum

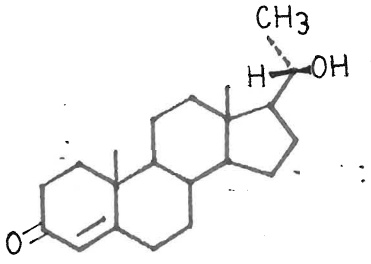
NAD(P)H:flavin oxidoreductase(I)

21-dehydroxylase(II)

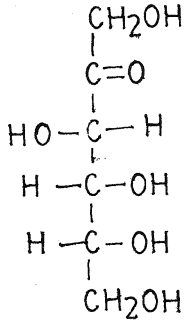
reported that growing cultures of E. lentum could not carry out 17α-dehydroxylation of steroid substrates. Therefore, the explanation for the inhibition by 17α-hydroxyprogesterone (211) must await further experiments.

The importance of an α-ketal structure ($R-\overset{O}{\overset{\|}{C}}-CH_2OH$) for 21-dehydroxylation was demonstrated[255] by the absence of a 21-dehydroxylated product from 20β,21-dihydroxy-Δ^4-pregnen-3-one (241) and by the lack of inhibition by this compound in mixed substrate competition experiments. Fructose (242) and dihydroxyacetone (243) did not inhibit the 21-dehydroxylation of deoxycorticosterone. This suggested that the 21-dehydroxylase may be restricted to compounds which also contain the perhydropentanophenanthrene nucleus (Fig. 1).

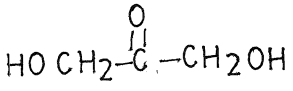
Kelley et al.[258] claimed that during the 21-dehydroxylation of deoxycorticosterone (244) labelled with ^{14}C at C4 and ^{3}H at both the C21 positions, more than half of the tritium was always exchanged in a mixed faecal flora incubation. On the basis of this finding, they suggested[258] that the 21-dehydroxylation of deoxycorticosterone (205) probably involves an enolization at C20,21. However, there was no confirmation that both the tritium atoms were located at C21 position of the substrate. Moreover, the $^{3}H/^{14}C$ isotopic ratio of the metabolites of this labelled deoxycorticosterone produced in their experiments was very low. The incubated labelled deoxycorticosterone had a $^{3}H/^{14}C$ ratio of 4.71, which itself is approaching to the limiting value for accurate $^{3}H/^{14}C$ ratio measurements. The metabolites of this labelled deoxycorticosterone has $^{3}H/^{14}C$ ratio as low as 0.08, a value too low for estimation within acceptable experimental



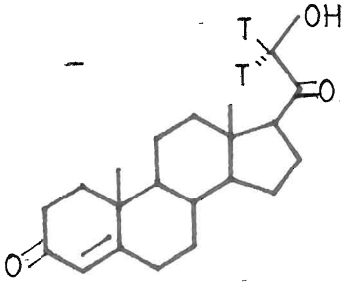
(241)



(242)



(243)



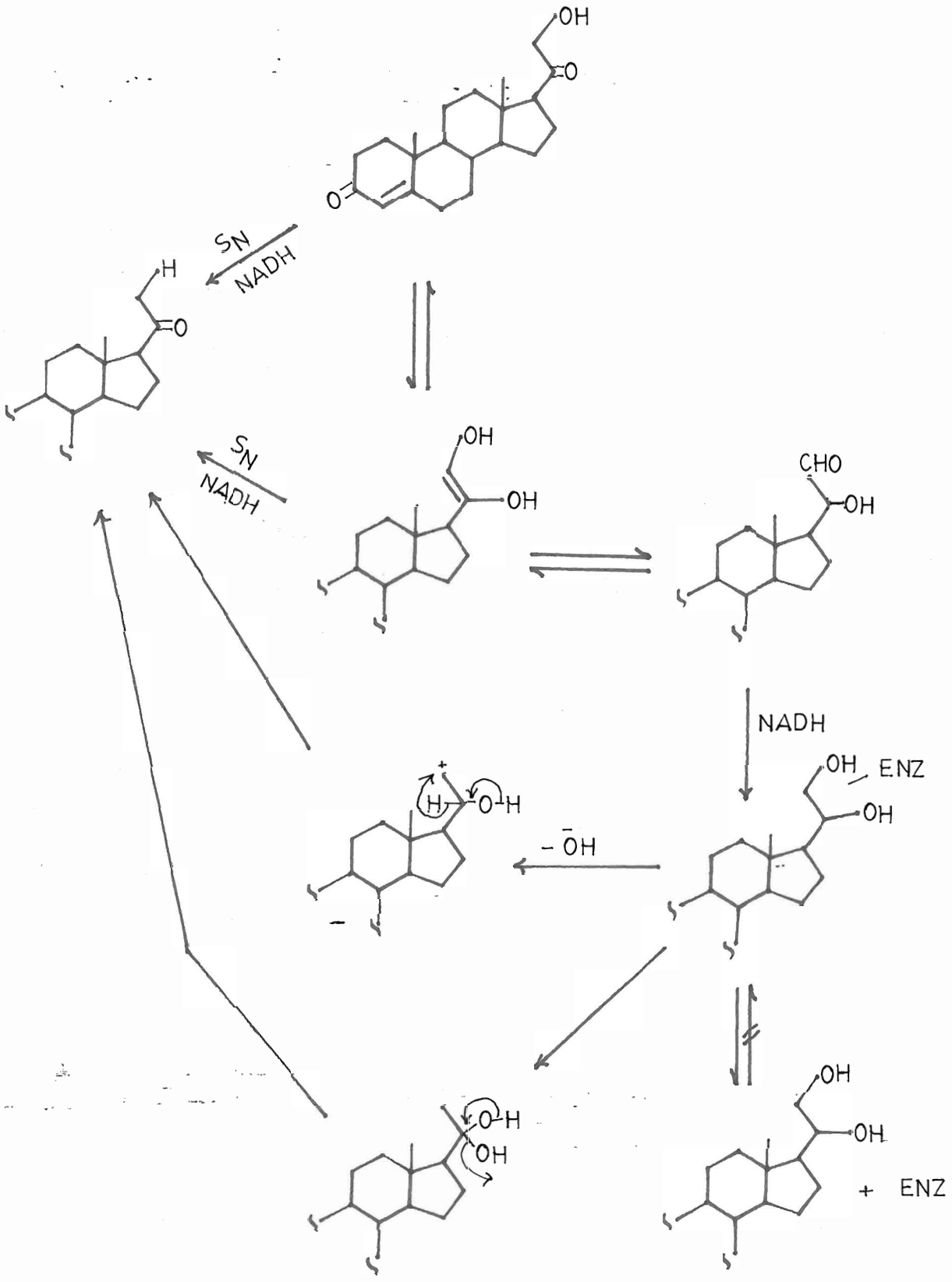
(244)

error limits. Moreover, Kelley et al.[250] did not report the re-isolation of the starting material, the tritium may therefore have been lost in the medium prior to the dehydroxylation.

Therefore, to have reasonable mechanistic information about 21-dehydroxylation, further investigation is desired. For this, we aimed to synthesize a 21-d_2 labelled deoxycorticosterone for incubation purposes. Some possible mechanisms for the 21-dehydroxylation of deoxycorticosterone are presented briefly in Figure 113. There are many advantages associated with C21-d_2 substrate and are as follows:

1. The label can be easily and accurately located in the substrate using ^2H NMR.
2. The amount of label at C21 position can be accurately estimated by comparing its relative amount with that at C17 position using ^2H NMR as both the deuterium signals differ significantly in their chemical shifts.
3. Any loss of label from the C21 position can be easily and accurately measured by mass spectrometry.
4. This labelled substrate may be used for measuring kinetic isotope effects which will provide useful mechanistic information. For example, if the 21-dehydroxylation occurs via C20-C21 enolization, a primary kinetic isotope effect (k_H/k_D) may be observed; on the other hand, if it occurs via an "S_N2" substitution, a secondary kinetic isotope effect will be observed.

Figure 113


OH
O
O
SN
NADH
H
O
SN
NADH
OH
OH
CHO
OH
NADH
OH
ENZ
OH
H
O
H
+
- OH
O
H
OH
OH
OH
+ ENZ

EXPERIMENTAL II

EXPERIMENTAL-II

Preparation of Labelled Steroids:

Ketalization of 11-deoxycorticosterone acetate

A solution of 11-deoxycorticosterone acetate (247) (50 g) in toluene (200 mL, dry) containing ethylene glycol (10 mL) was distilled (50 mL) to remove traces of water, then p-toluene sulfonic acid monohydrate (0.1 g) was added. The reaction mixture was refluxed overnight under anhydrous conditions with continuous removal of water using Dean Stark Separator. Then the reaction mixture was cooled to room temperature, potassium hydroxide solution (1.0 g in 20 mL MeOH) was added to it, and it was washed with water. The organic layer was separated and washings were thoroughly extracted with chloroform. The combined organic extract was dried over anhydrous sodium sulfate and evaporated *in vacuo* yielded a crude product (5.4 g). Fractional crystallization from ethyl acetate gave Δ^5-pregnen-20-one-21-ol-3,3-ethylene dioxyketal (250A) (2.30 g) m.p. 177°-179°C (lit.[260] 178-190°C), ir (KBr), ν_{max}: 3400 cm^{-1}, 1700 cm^{-1}. ^1H NMR data are given in Table 18.

Mass spec. m/z (%): 374 (M^+, 21.2), 359 (4.8), 346 (16.7), 343 (9.8), 331 (8.4), 315 (12.6), 299 (3.5), 286 (4.7), 271 (3.7), 253 (5.8), 229 (11.8), 99 (100).

On fractional crystallization also separated Δ^5-pregnen-3,20-dione-3,20-bis-ethylene-dioxyketal-21-ol (253) (0.90 g) m.p. 188-192°C from chloroform-petroleum ether (lit.[260] 188-193°C). ^1H NMR data are given in Table 18,

^{13}C NMR data are presented in Table 19.

Mass spec m/z (%): 418 (M^+, 4.6), 400 (3.2), 387 (100), 357 (10), 343 (18), 325 (16.5), 299 (6.1), 281 (89), 263 (12.1). (Ion abundances adjusted at m/z 387 as 100%.)

In one set of the experiment, Δ^4-progesten-3-one-21-ol-e-monoethylene dioxyketal (250B), m.p. 180-181°C (lit.[233] m.p. 180°C) was also obtained. ^1H NMR data are presented in Table, and ^{13}C NMR data in Table 19.

Mass spec. m/z (%): 374 (M^+, 20.3), 359 (4.1), 346 (15.9), 343 (8.8), 331 (8.4), 229 (12.3).

Hydrolysis of the mixture of bis- and mono-ketals of 11-deoxycorticosterone obtained as mixture from the ketalization of 11-deoxycorticosterone acetate

To a solution of the mixture of bis- and mono-ketals of 11-deoxycorticosterone (0.2 g) in water saturated benzene (25 mL) was added ethereal solution of p-toluenesulfonic acid monohydrate (2.5 mL, 0.01 M). The reaction mixture was stirred for 70 minutes at room temperature. Then it was diluted with benzene (50 mL), neutralized with 10% sodium bicarbonate solution (50 mL) and the organic layer was separated. The organic layer was washed with water (3 x 75 mL), dried over anhydrous sodium sulfate and evaporation in vacuo afforded a mixture of Δ^5-pregnen-3,20-dione-bis-ethylene dioxyketal (253), Δ^5-pregnen-3,20-dione-3-monoethylene ketal (250A) and Δ^4-pregnen-3,20-dione-20-monoethylene dioxyketal-21-ol (254) (indicated by ^1H NMR). The reaction was repeated several times with different reactions times, and using varying amounts of

p-toluenesulfonic acid, but the hydrolysis was not found to be specific towards the 20-ketal group.

Hydrolysis of 11-deoxycorticosterone-bis-ethylene dioxyketal (253):

To a solution of 11-deoxycorticosterone-bis-ethylene dioxyketal (253) (0.1 g) in water saturated benzene (25 mL), was added an ethereal solution of p-toluenesulfonic acid monohydrate (2.0 mL, 0.1 M). The resulting solution was stirred at room temperature and the reaction was monitored with tlc. No product formed after two hours, and the stirring was continued for a further six hours. It was diluted with benzene (75 mL), and neutralized with 10% sodium bicarbonate solution. The organic layer was separated, washed with water and dried over anhydrous sodium sulfate. Evaporation in vacuo gave a crude product (0.08 g). The ^{1}H NMR of the crude product showed the presence of deoxycorticosteron-20-ethylene dioxyketal (254) as indicated by the signal at δ 5.7 (s, C4, olefinic). The compound was not purified and was not employed in further studies.

Preparation of Δ^{5}-pregnen-20-one-3β-ol-17,21,21,21-d_4 (260):

To a solution of Δ^{5}-pregnen-20-one-3β-ol (214) (5.0 g) in benzene (100 mL, dry) containing tetrabutyl ammonium bromide (5.0 g) was added 5% NaOD solution (100 mL). The reaction was refluxed under conditions protected from atmospheric moisture for 48 hours. At the end of reaction, it was cooled to room temperature and the organic layer was separated, washed with pH 6.86 buffer (3 x 50 mL), dried over anhydrous sodium

sulfate, and evaporated in vacuo. The crude product (4.3 g) was crystallized from methanol to give Δ^5-pregnen-20-one-3β-ol-17,21,21,21-d_4 (260) (4.0 g) (79%), m.p. 189-190°C (lit.,[261] m.p. 190°C). The ^2H NMR spectrum (13.8 MHz) showed signals at δ 1.93 (br s, 3 x ^2H, C21 CD_3) and δ 2.13 (br s, 1 x ^2H, C17 ^2H), ^1H NMR data is presented in Table 18. Mass spec. m/z (%): 320 (M^+, 36.2), 203 (52.1), 287 (47.3), 264 (11.7), 256 (22.6), 244 (12.6), 213 (60.3), 209 (32.0), 199 (14.3), 191 (17.1). Mass spectral analysis showed the following isotopic composition: d_4 85.66%, d_3 14.33% (±1%).

Preparation of Δ^5-pregnen-20-one-3β-ol-17-d_1 (262):

To a solution of Δ^5-pregnen-20-one-3β-ol (214) (3.0 g) in dry benzene (100 mL) containing tetrabutylammonium bromide (2.0 g) was added 5% NaOD solution (50 mL), and the reaction mixture was refluxed for four hours. Workup as usual afforded the crude product (2.8 g) which on several crystallizations from methanol yielded Δ^5-pregnen-20-one-3β-ol-17-d_1 (262) (2.4 g) m.p. 190°C (lit.,[261] m.p. 190°C). The ^2H NMR spectrum (13.81 MHz) indicated signals at δ 2.009 (br s, 1 x ^2H, C17 ^2H). ^1H NMR data are presented in Table 18.

Mass spec. m/z (%): 317 (M^+, 16.1), 299 (12.1), 284 (13.2), 266 (4.5), 256 (5.4), 244 (3.9), 231 (9.2), 213 (26.1), 259 (14.6), 147 (10.8), 145 (20.5), 143 (15.7), 107 (24.3), 105 (29.3), 43 (100). The mass spectral analysis of molecular region m/z 319-313, showed the following isotopic composition: d_1 89.36%, d_0 10.63% (±1%).

Jones' Oxidation of Δ^5-pregnen-20-one-3β-ol-17,21,21,21-d_4 (261):

To a solution of Δ^5-pregnen-20-one-3β-ol-17,21,21,21-d_4 (261) (0.5 g) in acetone (25 mL) was added Jones' reagent (0.5 mL) dropwise with constant stirring at 0°C over a period of 15 minutes. The stirring was continued for an additional 10 minutes, then the excess reagent was destroyed by adding 2-propanol (2 mL), and the reaction was then diluted with ether (100 mL). The organic layer was separated, washed with pH 6.86 buffer (3 x 50 mL), dried over anhydrous sodium sulfate and evaporated *in vacuo*. The ^{1}H NMR spectrum of the crude product indicated the presence of Δ^4-3-one (conjugated) and Δ^5-3-one (unconjugated) species. The detailed mass spectrum in the molecular ion region showed the presence of deuterated species d_1, d_2, d_3 and d_4 with undetermined loss. This product was not used in further reactions.

Oppenauer oxidation[262] of Δ^5-pregnen-20-one-3β-ol-17,21,21,21-d_4 (260):

A solution of pregnenolone-d_4 (260) (1.0 g) in toluene (150 mL, dry) containing cyclohexanone (7 mL) was distilled (50 mL) to remove the traces of water, then the freshly distilled aluminum isopropoxide (0.4 g) was added and the reaction mixture was slowly distilled over four hours. When most of the toluene was distilled out, the reaction mixture was cooled to room temperature and a solution of sodium potassium tartarate (20 mL, 0.1 M) was added. The cyclohexanol and cyclohexanone were removed by steam distillation and the reaction mixture was thoroughly extracted with ether. The organic extract was washed with pH 6.86 buffer (3 x 50 mL), dried over anhydrous sodium sulfate and evaporated *in vacuo*. Crystallization

from ethyl acetate-hexane a crude product of m.p. 120°C. The detailed mass spectral analysis in the molecular region indicated that considerable exchange of deuterium atoms. Therefore, this labelled progesterone was not used in further experiments.

In another Oppenauer oxidation using aluminum t-butoxide,[90] considerable loss of deuterium was also observed.

In a third Oppenauer oxidation using aluminum t-butoxide[90] and acetone-d_6 (as hydrogen acceptor) were used. The mass spectral analysis in the molecular ion region indicated the presence of appreciable amount of d_5 species along with other deuterated species, showing random exchange loss of deuterium. This labelled progesterone was also not used in further studies.

Δ^5-Pregnen-3,20-dione-3,20-bis-ethylene dioxyketal (265)[269]

A mixture of progesterone (213) (15.0 g), ethylene glycol (300 mL) and p-toluenesulfonic acid monohydrate (0.45 g), while stirring was distilled at slow rate over two hours at 1.5 mm Hg pressure (still head temperature 77-81°C). The reaction mixture became turbid in about 30 minutes and crystals separated after 45 minutes. A purple colour developed after one hour. The reaction mixture was then made alkaline with alcoholic potassium hydroxide solution and poured onto equal volume of water. The solid collected after filtration was washed with water and a crude product (18.9 g) was obtained. Recrystallization from acetone afforded Δ^5-pregnen-3,20-dione-bis-ethylene dioxyketal (265) (12.5 g), m.p. 180-182°C

(lit.,[263] m.p. 180-183°C). On concentrating the mother liquor, another 2.1 g of the product was obtained. ir (nujol) ν_{max}: no carbonyl absorption band. ^{1}H NMR data are given in Table 18, and ^{13}C NMR data are presented in Table 19.

Mass spec. m/z (%): 402 ($M^{+\cdot}$, 1.6), 358 (1.0), 338 (0.7), 312 (2.0), 272 (1.3), 229 (1.1), 99 (100), 91 (6.0), 87 (42.8).

Δ^5-Pregnen-3,20-dione-3-monoethylene dioxyketal (266)[264]

To a solution of progesterone-bis-ethylene ketal (265) (4.0 g) in benzene saturated with water (500 mL) was added the solution p-toluene-sulfonic acid monohydrate (100 mL, 0.01 M in ether) and the resulting reaction mixture was stirred for 1.5 hours at room temperature. At the end of reaction, it was diluted with benzene (250 mL) and neutralized with 10% sodium bicarbonate solution (250 mL). The organic layer was separated, washed with water (4 x 100 mL), dried over anhydrous sodium sulfate and evaporated to dryness in vacuo. Crystallization from methanol afforded Δ^5-pregnen-3,20-dione-3-ethylene dioxyketal (266) (3.5 g, 98%), m.p. 179-180°C (lit.,[265] m.p. 180-181°C), ir (nujol) ν_{max}: 1700 cm^{-1}. ^{1}H NMR data are given in Table 18, and ^{13}C NMR data are presented in Table 19.

Mass spec. m/z (%): 358 ($M^{+\cdot}$, 2.8), 338 (1.4), 314 (2.2), 312 (2.3), 272 (2.6), 229 (3.0), 173 (2.3), 159 (3.0), 147 (3.5), 124 (4.4), 99 (100).

Δ^4-Pregnen-3,20-dione-3-ethylene dioxyketal-17,21,21,21-d_4 (267):

To a solution of progesterone-3-monoethylene ketal (266) (2.0 g) in benzene (100 mL, dry) containing tetrabutylammonium bromide (1.0 g) was added 5% NaOD solution (40 mL, 2.0 g sodium metal in 40 mL D_2O). The resulting reaction mixture was refluxed under conditions protected from atmospheric moisture for four hours. At the end of the reaction, the resulting reaction mixture was cooled to room temperature and the organic layer was separated. This organic layer was washed with pH 6.86 buffer solution (3 x 100 mL), dried over anhydrous sodium sulfate and evaporated to dryness in vacuo. Crystallization from benzene-hexane afforded Δ^5-pregnen-3,20-dione-3-ethylene dioxyketal-17,21,21,21-d_4 (267) (1.8 g) m.p. 180°C (lit.,[265] m.p. 180-181°C, unlabelled), ir (nujol) ν_{max}: 1700 cm^{-1}. The ^2H NMR spectrum (13.8 MHz) showed signals at δ 1.885 (br s, 3 x ^2H, C21 CD_3) and 2.15 (s, shoulder, 1 x ^2H, C17 ^2H). Mass spec. m/z(%): 362 (M^+, 4.3), 318 (2.1), 276 (1.1), 2.4 (5.9), 99 (100), 91 (4.2), 55 (10.0), 46 (19.5). The mass spectral analysis of the molecular ion region (m/z 364-360) indicated the following isotopic composition: d_4 97.68%, d_3 3.02%, $d_2 = d_1 = d_0 = 0\%$ (±1%).

Δ^5-Pregnen-3-one-20β-ol-3-ethylene dioxyketal-17,21,21,21-d_4 (268)[266]

To a three-necked round bottom flask containing THF (100 mL, dry) equipped with magnetic stirrer and condenser, and maintained under the atmosphere of dry nitrogen, was added $LiAlH_4$ powder (0.26 g). A solution of Δ^5-pregnen-3,20-dione-3-cycloethylene ketal-d_4 (267) (2.4 g) in THF

(50 mL, dry) was added dropwise using a pressure equalizer dropping funnel, to the above $LiAlH_4$ suspension, over 30 minutes while stirring. The resulting mixture stirred for additional 30 minutes, then the excess reagent was destroyed with a few drops of ethyl acetate and the reaction filtered. The precipitate thus obtained was dissolved in 10% sulfuric acid solution (10 mL) and then thoroughly extracted with chloroform. The chloroform extract was washed with water (2 x 50 mL) and pH 6.86 buffer solution (2 x 50 mL). The combined organic extract was dried over anhydrous sodium sulfate and evaporated to dryness in vacuo. Crystallization from acetone afforded Δ^5-pregnen-3-one-20β-ol-3-ethylene dioxyketal-17,21,21,-21-d_4 (268) (2.1 g) slightly contaminated by Δ^5-pregnen-3-one-20α-ol-3-ethylene dioxyketal-17,21,21,21-d_4 (273). This mixture was not purified and was utilized in further reactions.

Δ^4-Pregnen-3-one-20β-ol-17,21,21,21-d_4 (269):

The crude product obtained from the above reaction was dissolved in chloroform (100 mL), 36% HCl solution (10 drops in 3 mL of methanol) was added to it, and the resulting solution was stirred at room temperature for 6 hours. At the end of the reaction, it was neutralized with 5% NaOH solution, washed successively with water (4 x 50 mL), pH 6.86 buffer solution (2 x 50 mL) and finally with saturated sodium chloride solution. The chloroform layer was dried over anhydrous sodium sulfate and evaporated to dryness in vacuo. Crystallization from ethylene acetate-hexane afforded mainly Δ^4-pregnen-3-one-20β-ol-17,21,21,21-d_4 (269) (1.57 g), m.p. 170-173°C

170-173°C (lit.,[267] 172-175°C, unlabelled), ir (nujol) ν_{max}: 3580, 2090, 1680 cm^{-1}. ^1H NMR data are given in Table 18.

Mass spec. m/z (%): 320 (M^+, 10.4), 203 (6.8), 287 (3.4), 278 (13.2), 269 (7.3), 260 (6.61), 231 (12.6), 230 (15.1), 196 (8.3), 179 (18.1), 177 (13.8), 150 (25.9), 124 (100).

Δ^4-Pregnen-3,20-dione-17,21,21,21-d_4 (261):

To a solution of Δ^4-pregnen-3-one-20β-ol-d_4 (269) (1.2 g) in acetone (100 mL), cooled to 0°C, was added dropwise Jones' reagent (1.32 mL) with constant stirring in the course of one hour. The resulting reaction mixture was stirred for additional 10 minutes, then the excess of the reagent was destroyed with 2-propanol (2 mL) and diluted with water (100 mL). Ether (100 mL) was added to the above reaction mixture and the organic layer was separated. The aqueous layer was thoroughly extracted with ether (3 x 50 mL). The combined ethereal extract was washed with pH 6.86 buffer solution (2 x 50 mL), dried over anhydrous sodium sulfate and evaporated to dryness in vacuo. Crystallization from benzene-petroleum ether (60-110°) yielded Δ^4-pregnen-3,20-dione-17,21,21,21-d_4 (261) (1.10 g), m.p. 127-128°C (lit.,[268] 128°C). The ^2H NMR spectrum (13.81 MHz) included signals at δ 1.903 (br s, 3 x ^2H, C21 CD_3) and 2.168 (br s, 1 x ^2H, C17 ^2H). ^1H NMR data are presented in Table 18.

Mass spec m/z (%): 318 (M^+, 18.6), 303 (4.1), 276 (26.4), 244 (10.3), 230 (17.7), 195 (12.9), 124 (88.6), 91 (40.9), 67 (20.7), 46 (100). The mass spectral analysis of molecular region (m/z 320-314) showed the following isotopic composition: d_4 85.8%, d_3 6.4%, d_2 0%, d_1 4.8%, d_0 3.9% (±1%).

Incubations with Aspergillus niger ATCC-9142

Aspergillus niger ATCC-9142 was obtained from American Type Culture Collection, Washington, D.C., and maintained on 4% malt agar slopes. The slopes were recultured after five to six week intervals.

Incubations in Czapek Dox nutrient medium:[269]

The medium consisted of Czapek Dox nutrient (33.4 g) dissolved in distilled water (1 L). Incubations were performed in one-liter Erlenmeyer flasks each containing 150 mL of the liquid medium. The flasks were sterilized at 15 pounds pressure at 120°C for 15 minutes. After cooling to the room temperature, the flasks were inoculated with A. niger, and were then incubated on a New Brunswick rotary shaker at about 180 rpm for three to four days at room temperature. The steroidal substrates were then added in 95% ethanol (50 mg/mL) and incubation was continued for four days. The mycelia were separated from the medium, macerated in Waring blender with CH_2Cl_2. Both the mycelium and medium were extracted thoroughly with CH_2Cl_2. The methylene chloride extract was washed with distilled water, saturated sodium chloride solution, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was then subjected to chromatography when required.

Incubation of Δ^4-pregnen-3,20-dione-17,21,21,21-d_4 (261):

The progesterone-d_4 (261) (0.5 g) was incubated in ten, one Liter Erlenmeyer flasks, each containing Czapek-Dox nutrient (33.4g/L) medium

and three slopes were used for inoculation. This incubation gave a crude extract (0.43 g) which was chromatographed on silica gel column. Elution with benzene-ether (90:10) gave starting material (0.210 g), further elutions gave slightly impure Δ^4-pregnen-3,20-dione-21-ol-17,21,21-d_3 (0.05 g). The ^2H NMR spectrum showed loss of deuterium from the C21 carbon, only half of the original deuterium remained, which was distributed equally between the diastereotopic C21 positions. ^2H NMR (61.402 MHz) spectrum showed signals at δ 1.82 (^2H, C17); 3.87, 4.0 ($\frac{1}{2}$ ^2H, C21).

Incubation of progesterone (213):

Progesterone (0.3 g) was incubated as described previously, in three, one liter Erlenmeyer flasks. After three days of mycelial growth, the growth medium (Czapek-Dox nutrient) had pH 3.2. In one flask, the medium was replaced by distilled water (150 mL), while in a second flask, it was replaced by pH 6.86 buffer (7 g + 200 mL). All the three flasks were incubated with progesterone (0.1 g each in ethanol) and mycelial growth was allowed to continue for a further four days. After separating the mycelium from the medium, the pH of each flask was measured and is as follows:

1. Replacement with distilled water pH 2.5
2. Replacement with pH 6.86 buffer solution pH 3.3
3. Without replacement of growth medium pH 2.3

The ^1H NMR spectrum of the crude product (from the incubation flask where the growth medium was replaced by distilled water) showed a 30%

formation of 11-deoxycorticosterone. This conversion was the best achieved.

No metabolism of progesterone was obtained when $CaCO_3$ was suspended in one of the incubation flasks where the growth medium was replaced with distilled water after three days of mycelial growth.

Incubations using growth medium #2:

Growth medium #2: In 1.0 Liter of distilled water was added the following:

sucrose (20 g)

peptone (40 g)

corn steep liquor (2 mL) (Grand Island Biochemical Co.).

The above liquid medium was boiled to dissolve the components, and 150 mL of the resulting solution was transferred to each 1.0 Liter Erlenmeyer flask and autoclaved (20 minutes, at 20 pounds pressure, 120°C). A spore suspension of A. niger was then transferred into two of these flasks. After three days of mycelial growth, the growth medium had pH 4.3. In one of the flasks, the growth medium #2 was replaced with distilled water, and washed mycelia were resuspended in it. A solution of progesterone (213) (0.1 g/mL) was added to each flask and fungal growth was allowed to continue for a further ~4 days. At the end of incubation period, the mycelium was separated from the medium and pH was measured.

1. Incubation in growth medium #2 pH 2.7-2.9
2. Incubation after replacement with distilled water pH 2.5

After extraction as usual, the ^1H NMR spectrum showed a 60% conversion of progesterone in the incubation with growth medium #2, and a 50% conversion in distilled water replacement culture growth.

Incubations using growth medium #3:

Growth medium #3: In 1.0 Liter of the distilled water was added the following:

malt extract (20.0 g)

peptone (4.0 g)

corn steep liquor (5.0 mL)

The liquid medium #3 after dissolving was autoclaved as described previously and 150 mL of the medium was transferred to each 1.0 Liter Erlenmeyer flask. After 3 days of fungal growth pH of the medium was found to be 4.2, and on the fourth day it was decreased to 3.2. On the fourth day, progesterone (261) (0.2 g/2 mL) was incubated equally in the original medium and in the flask where the medium was replaced by distilled water (150 mL). The fungal growth was allowed to continue for an additional four days, and then the mycelium and medium were separated by filtration. The pH of each medium was then measured, and is as follows:

1. Incubation in growth medium #3 pH 2.8
2. Incubation after replacement with distilled water pH 2.5

The ^{1}H NMR spectra of crude products (from the above two incubations) indicated a 20% conversion of progesterone in each of the incubations.

Table 18. ^{1}H NMR data

Compound	C3	C4	C6	C18	C19	C20	C21	extra
DOC (205)	--	5.73 (s, 1H, olefinic)	--	0.68 (s, 3H)	1.08 (s 3H)	--	4.18 (br s, 2H)	--
Δ^4-DOC-3-monoketal (250B)	--	5.3 (s, 1H, olefinic)	--	0.65 (s, 3H)	1.02 (s, 3H)	--	4.18 (br s, 2H)	3.95 (s, 4H, ketal)
Δ^5-DOC-3-monoketal (250A)	--	--	5.25 (br s, olefinic)	0.65 (s, 3H)	1.02 (s, 3H)	--	4.18 (br s, 2H)	3.95 (br s, 4H, ketal)
DOC-bisketal (253)	--	--	5.30 (br d, 1H, J 6.0 Hz)	0.78 (s, 3H)	1.0 (s, 3H)	--	3.49 (br s, 2H)	3.2 (br s, 1H, OH) 3.95 (br s, 8H, ketal)
pregnenolone-d_1 (262)	3.15-3.82 (br m, $1H_\alpha$)	--	5.33 (d, 1H, olefinic, J 5.0 Hz)	0.65 (s, 3H)	1.01 (s, 3H)	--	2.12 (s, 3H)	
pregnenolone-d_4	3.15-3.82 (br m, 1H)	--	5.33 (d, 1H, olefinic J 5.0 Hz)	0.65 (s, 3H)	1.01 (s, 3H)	--	2.13 (s, 3H)	--
Progesterone-d_4 (261)	--	5.75 (s, 1H olefinic)	--	0.67 (s, 3H)	1.09 (s, 3H)	--	--	--

Table 18. (continued)

Compound	C3	C4	C6	C18	C19	C20	C21	extra
Δ^4-pregnen-3-one-20β-ol-d_4 (269)	--	5.75 (s, 1H, olefinic)	--	0.8 (s, 3H)	1.19 (s, 3H)	3.73 (br s, 1H)	--	--

br = broad

m = multiplet

s = singlet

d = doublet

Table 19. ^{13}C NMR data

Carbon number	(253)	(250B)	(266)	(265)
1	36.3	35.8	36.4	36.3
2	31.5*	30.0	31.5*	31.5*
3	109.5	106.1	109.4	109.5
4	41.6	120.1	41.8	41.9
5	140.2	151.1	140.3	140.3
6	122.0	34.9	121.9	122.1
7	31.6*	31.1	31.7*	31.7*
8	31.0	31.9	31.9	31.1
9	49.7	53.8	49.7	49.8
10	36.6	35.8	36.7	36.7
11	20.8	21.0	21.1	20.9
12	39.2	38.6	38.9	39.5
13	41.6	44.8	44.0	41.9
14	56.5	56.4	57.0	56.7
15	23.9	24.5	24.5	24.5
16	22.8	23.0	22.9	23.1**
17	53.4	59.2	63.7	58.2
18	13.0	13.4	13.3	12.9
19	18.9	17.6	18.9	18.9
20	112.6	210.4	209.4	112.0
21	66.2	69.4	31.1	23.9**
ketals	64.2 64.4(2) 65.5	64.2 66.6	64.3 64.4	64.2 64.4 63.3 65.2

* chemical shifts may be interchanged

** chemical shifts may be interchanged

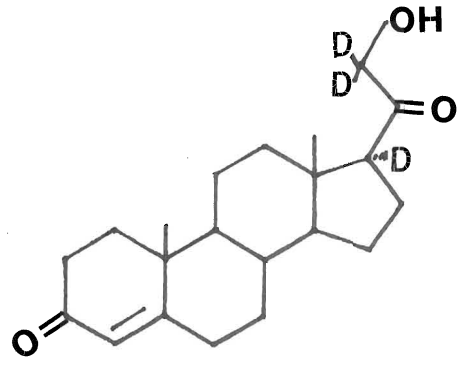
RESULTS AND DISCUSSION--II

RESULTS AND DISCUSSION--II

Preparation of labelled steroids

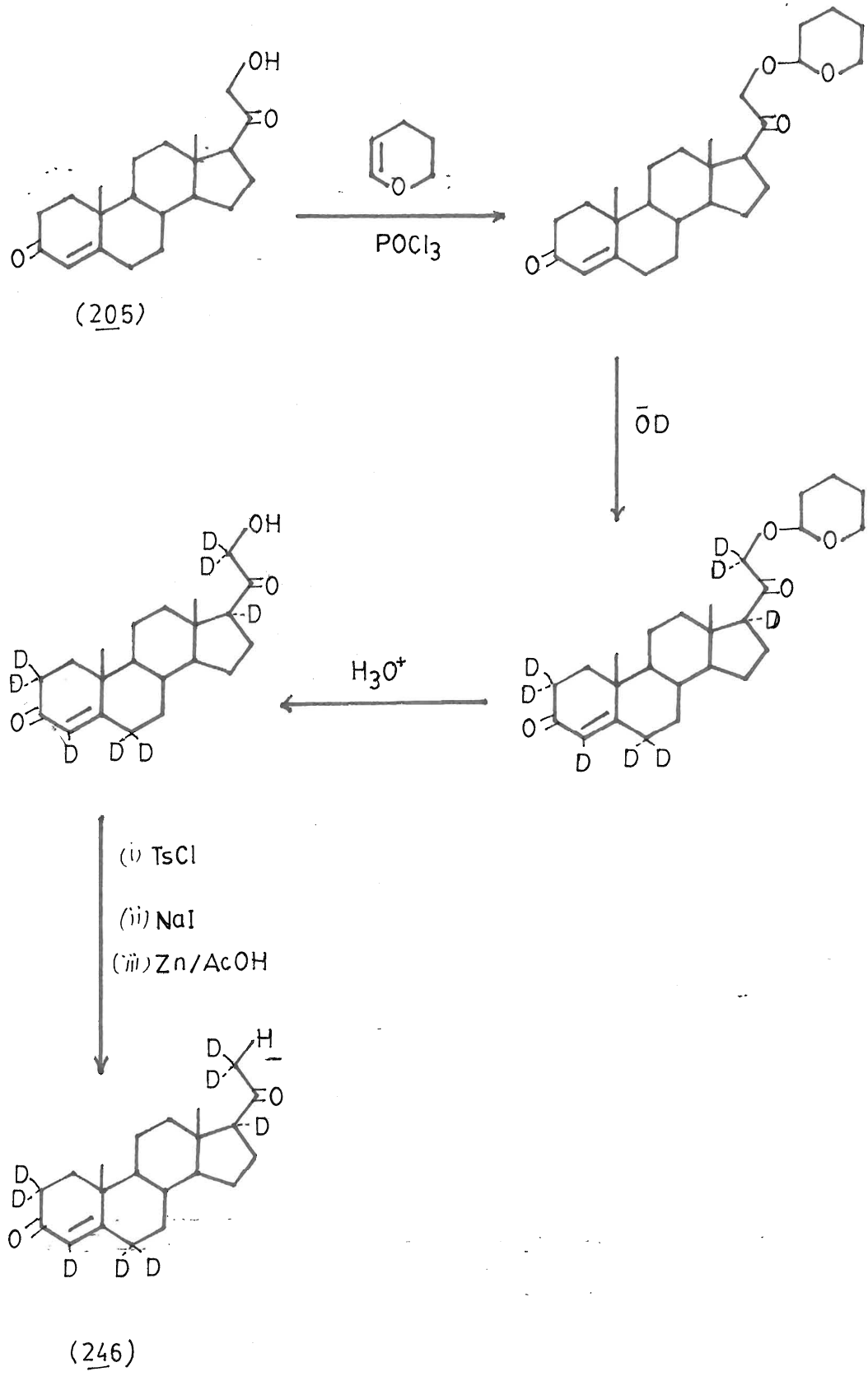
The major synthetic difficulty encountered during the preparation of deuterium-labelled substrates was the loss of label due to enolization. Several synthetic schemes were designed and attempted to introduce maximum label at the enolizable positions. Deoxycorticosterone-17,21,21-d_3 (245) was the desired labelled substrate for its 21-dehydroxylation studies.

Holland and Auret[269] have successfully employed a scheme (Fig. 114) in synthesizing 2,2,4,6,6,17,21,21-d_8 progesterone (246) for its use in the mechanistic study of 21-microbial hydroxylation of progesterone. Inspired by that, a closely analogous scheme (Fig. 115) was designed, though it was found not very useful for present study. In the very first attempt to prepare Δ^5-pregnen-3,20-dione-21-ol-21-acetate-3,20-bis-ethylene dioxyketal (deoxycorticosterone acetate-bis-ketal) (248), the 11-deoxycorticosterone acetate (247) was refluxed with ethylene glycol and p-toluene sulfonic acid in toluene (dry, from an old bottle) and water formed was continuously removed by Dean-Stark separator. This surprisingly afforded a very pure sample of Δ^5-pregnen-3,20-dione-21-ol-3-ethylene ketal (250A). The possibility of deoxycorticosterone acetate-bis-ethylenedioxy ketal (248) was discarded by spectral means. The infrared spectrum showed absorption band at 3400 cm^{-1} for 21-OH group and at 1700 cm^{-1} for C20 carbonyl group. The ^1H spectrum included signals at δ5.40-5.76 (m, 1H, olefinic proton at C6), 4.18 (br s, 2H, C21), 4.0 (s, 4H, O-CH_2-CH_2-O), and had m.p. 177-180°C in good agreement with the literature[260] value (m.p. 178-190). Then



(245)

Figure 114

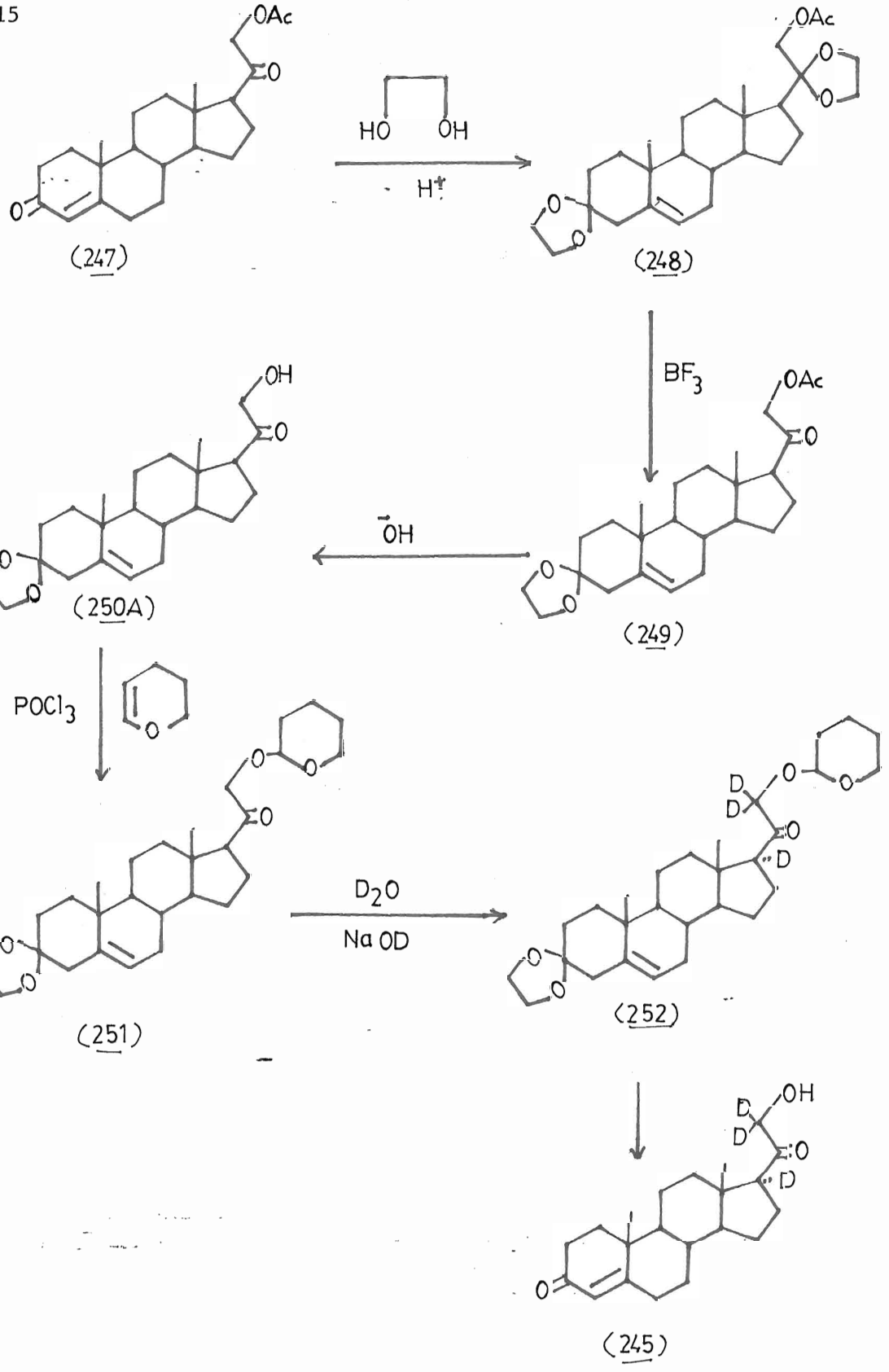


(205)

(246)

Scheme-I

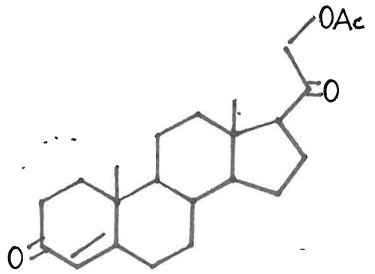
Figure 115



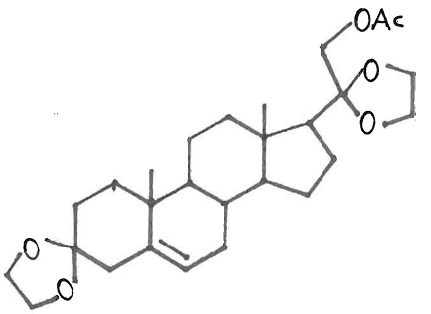
as expected, mass spectrum showed the molecular ion at m/z 374 (12%), and the fragment ion at m/z 315 (18%) by losing the C17 side chain ($-CO-CH_2OH$). However, when these results were found not reproducible when freshly dried toluene was used. The procedure was repeated with varying reflux timings, using varying amounts of p-toluenesulfonic acid and with dry benzene also. In most of these experiments, Δ^5-pregnen-3,20-dione-21-ol-3,20-bis-ethylene ketal (253) and deoxycorticosterone-3-monoethylene ketal (250A) were formed as major products. The ^1H NMR spectrum of the crude products also indicated the presence of 21-acetate derivatives in small amounts. In one set of these experiments, Δ^4-deoxycorticosterone-3-ethylenedioxy ketal (250B) was also isolated from the reaction mixture.

The important observation from this series of experiments was the unusual acidic hydrolysis of 21-acetate group. This hydrolysis most probably involves the particicpation of 20-keto group and the involvement of H_2O produced from the ketalization of 3-keto group. A probable mechanism for this hydrolysis is presented in Figure 116. Deoxycorticosterone bis-ketal (253) was probably formed after the hydrolysis of C21 acetate group. This may also account for the formation of deoxycorticosterone-3-monoethylene ketal (250A) as a major product.

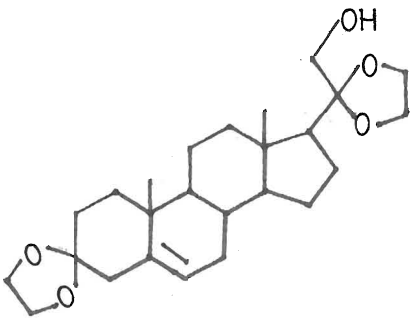
Antonucci et al.[270] reported the preparation of Δ^5-pregnen-3,20-dione-21-ol-21-acetate-3-ethylene ketal (249) from deoxycorticosterone acetate (247) in 40% yield by refluxing in benzene with ethylene glycol and p-toluenesulfonic acid. The steric crowding due to 21-acetoxy group in deoxycorticosterone acetate (247) was most probably responsible for the low reactivity of the C20 carbonyl group.



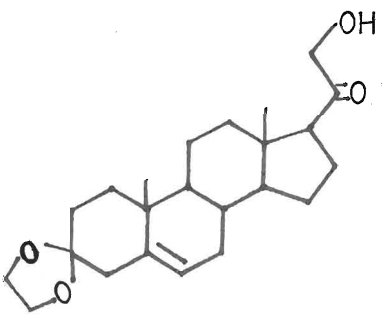
(247)



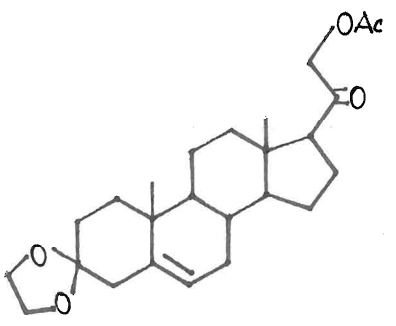
(248)



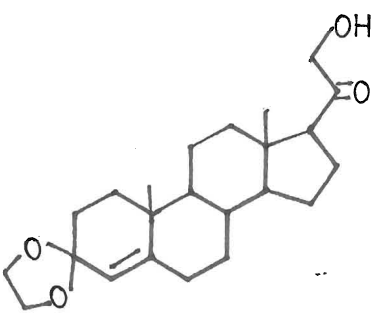
(253)



(250A)

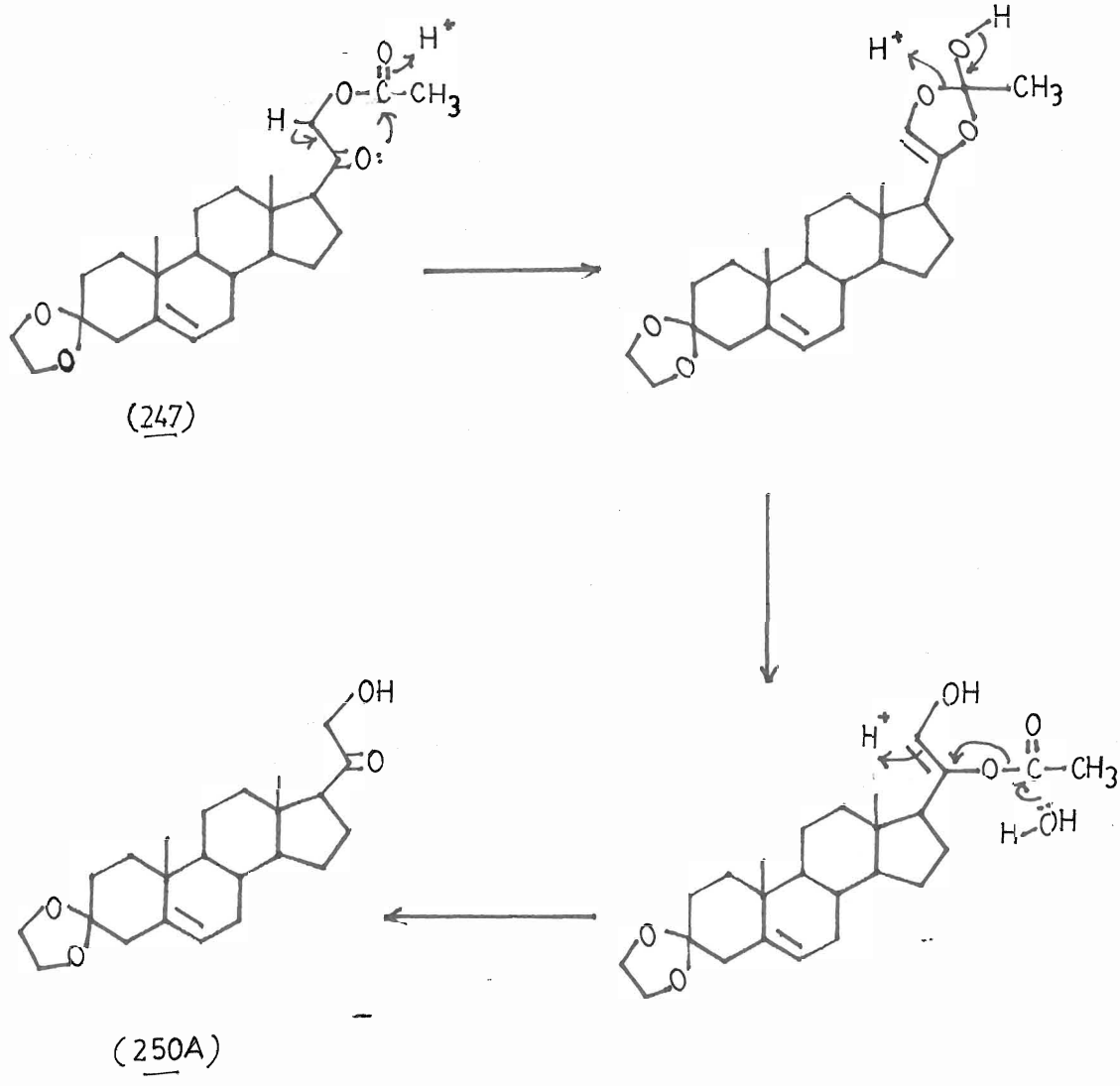


(249)



(250B)

Figure 116



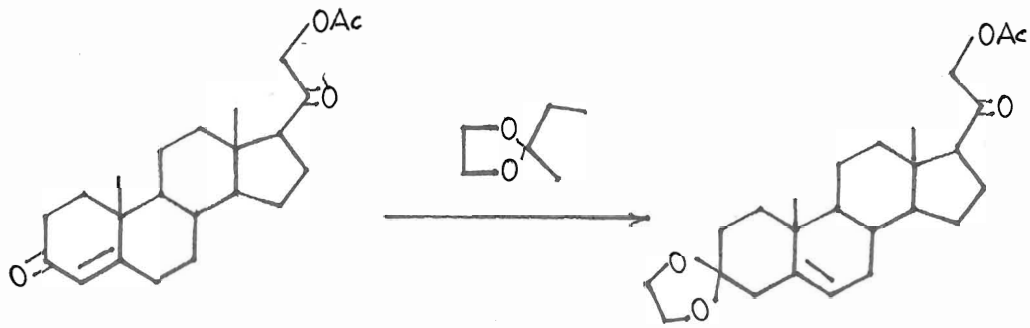
Bowers et al.[273] reported the synthesis of deoxycorticosterone acetate-3-monoethylene ketal (249) using the more hindered protecting group, dioxolane[274] (ethylenedioxy ketal of ethylmethyl ketone) (Fig. 117).

Since the present scheme (Fig. 115) for the synthesis of labelled deoxycorticosterone (245) in fact, did not require the protection of C20 carbonyl group, a pure sample of deoxycorticosterone-bis-ethylene dioxyketal (250A) was used for partial hydrolysis of C20 ketal group. This was an attempt to see if the crude product from the ketalization reaction of deoxycorticosterone acetate (247) consisted of mainly deoxycorticosterone-3-monoethylenedioxy ketal (250A) and deoxycorticosterone-bis-ethylenedioxy ketal (253), can be directly used to prepare the desired one (250A) without chromatographic separation of the mixture. After two hours stirring deoxycorticosterone-bis-ethylenedioxy ketal (253) with p-toluenesulfonic acid (0.1 M in ether) in benzene saturated with water, no hydrolysis occurred. When the stirring was continued for longer period of time, C3 ketal group was selectively hydrolyzed to give deoxycorticosterone-20-monoethylenedioxy ketal (254) (Fig. 118). This was indicated by the ^{1}H NMR spectrum of the crude product, showing a singlet at δ 5.7 for a C4 olefinic proton.

Sondheimer and Klibansky[275] have reported that deoxycorticosterone acetate-bis-ethylenedioxy ketal (248) on hydrolysis with p-toluenesulfonic acid in acetone was selectively hydrolysed to deoxycorticosterone acetate-20-monoethylenedioxy ketal (255) (Fig. 119).

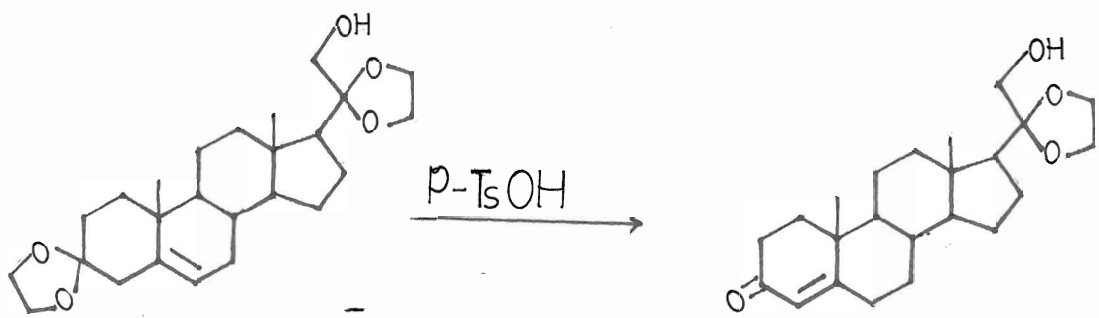
Δ^4-Pregnene-3,21-diol-20-one-20-ethylene dioxyketal (256)[275] was hydrolyzed only 50% with p-toluenesulfonic acid in ethanol even after six days. Its

Figure 117



(247) (249)

Figure 118



(253) (254)

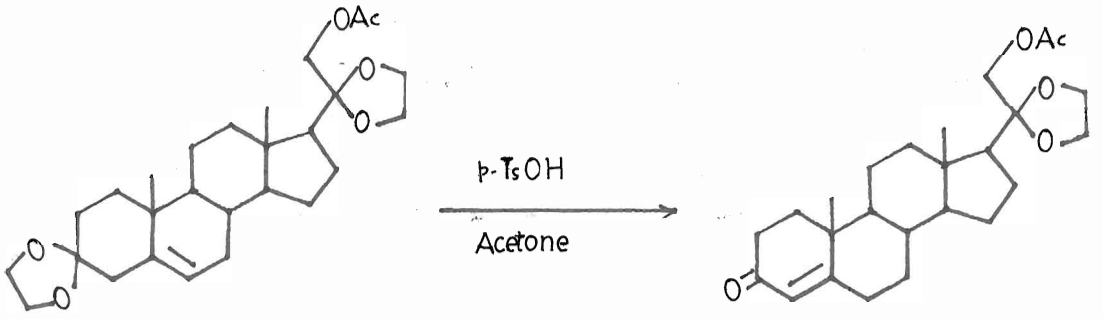
presence was indicated by the infrared spectrum of the crude product (257) (Fig. 120).

The present findings, along with Sondheimer and Klibansky's[275] observations, suggest that the hydrolysis of C20 ketal group is more difficult and the reactions non selective due to the steric crowding caused by the hydroxyl or acetoxy group at C21 carbon. Therefore, in the presence of ketals at both the C3 and C20 positions, the C3 ketal is more susceptible and selectively hydrolysed to the corresponding 3-keto group, as it is unhindered and more exposed to the reagent.

The scheme represented in Figure 115 was abandoned without any further progress on it. Another concern about this scheme was that the 21-(2'-tetrahydropyranyl) ether derivative (252) may lose some label during its conversion to the 21-hydroxy-3,20-dione derivative (245) during hydrolysis. In fact, under the conditions desirable for the above hydrolysis, deuterium loss has been observed.[269] During the hydrolysis of 21-(2'-tetrahydropyranyl) ether-d_8 (258) to the corresponding 3,20-dione-d_8 (259), loss of label was observed (Fig. 121). The deuterium isotopic composition of (258) was d_8 74%, d_7 18%, d_6 7%, d_5 1%, and after the acidic hydrolysis the product deoxycorticosterone-d_8 (259) had the isotopic composition of d_8 41%, d_7 39%, d_6 14%, d_5 6%.

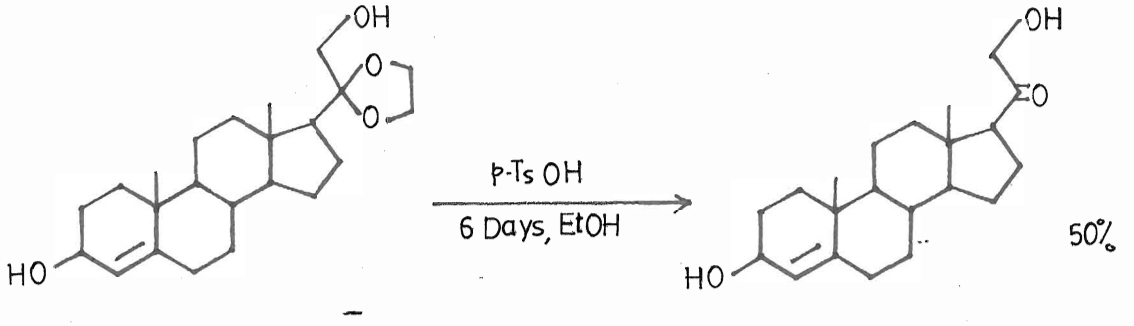
With the observed failure, another Scheme II (Fig. 122) was designed and undertaken. This scheme involved the synthesis of labelled progesterone (261) which could be converted later into the correspondingly labelled deoxycorticosterone (245) by enzymatic hydroxylation.

Figure 119

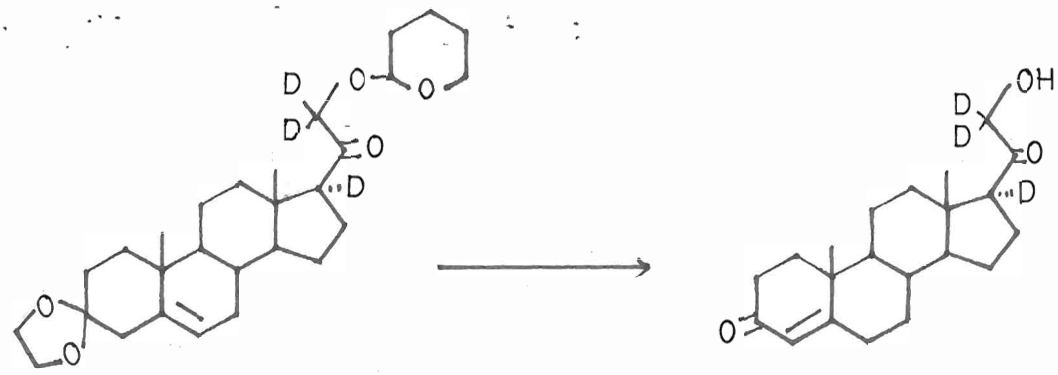


(248) (255)

Figure 120

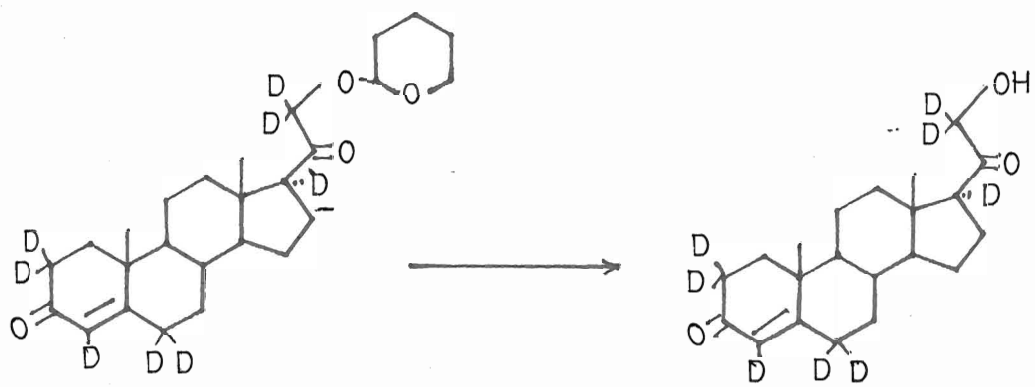


(256) (257)



(252) (245)

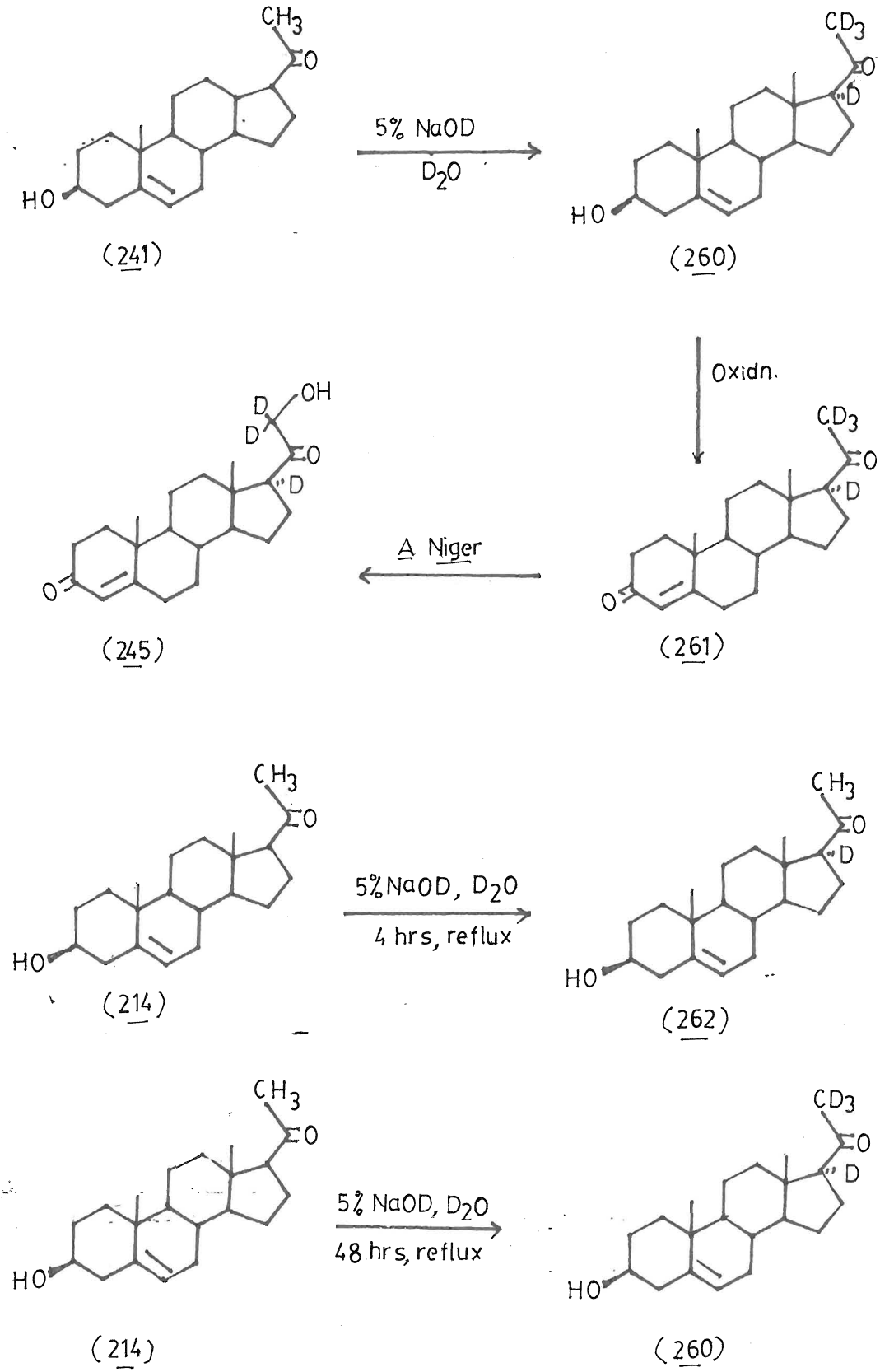
Figure 121



(258) (259)

Figure 122

Scheme-II



On refluxing, a solution of Δ^5-pregnen-3β-ol-20-one (pregnenolone) (214) in dry benzene with 5% NaOD using tetrabutylammmonium bromide as the phase transfer catalyst, for four hours, afforded Δ^5-pregnen-3β-ol-20-one-17d_1 (262). The incorporation of only one deuterium atom at C17 position was confirmed from its ^2H NMR spectrum indicating only one signal at δ 2.009, and the mass spectrum. The examination of ions at m/z 319-313 revealed the molecular isotopic composition of d_1 89.36 %, d_0 10.63%.

This labelled pregnenolone (262) was not the desirable compound for the present investigation. The main aim of the present study was the introduction of deuterium at both the C21 positions in deoxycorticosterone.

In another set of experiments, when the refluxing was extended to 48 hours, the desired Δ^5-pregnen-3β-ol-20-one-17,21,21,21-d_4 (pregnenolone d_4) (260) was obtained in 79% yield. The mass spectral analysis of ions at 322-316 gave the isotopic composition of d_4 85.66%, d_3 14.33, d_2 d_1 0%, and the ^2H NMR spectrum indicated signals at δ 2.13 (s, 1^2H, C17$^2H\alpha$) and 1.93 (s, 3^2H, C21 CD_3). The mass spectral fragmentation was also in good agreement with the structure (260).

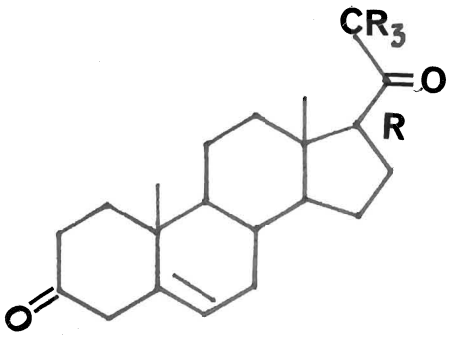
The next step in the envisaged Scheme II was the oxidation of 3β-OH group to 3-ketonic group together with isomerization of $\Delta^{5(6)}$ double bond to $\Delta^{4(5)}$ position.

Djerassi et al.[276] have reported the oxidation of pregnenolone to Δ^5-pregnene-3,20-dione (263) using Jones reagent in 89% yield. Therefore, it was hoped that this reaction may be employed for the oxidation of pregnenolone d_4 (260) to progesterone d_4 (261) by using a slight excess of the reagent for isomerizing the $\Delta^{5(6)}$ double bond to the $\Delta^{4(5)}$ position.

In an attempt to oxidize pregnenolone d_4 (260) to progesterone d_4 (261) using a slight excess of Jones reagent at 0°C in dry acetone, a mixture of two products was obtained. The ^1H NMR spectrum of the crude product indicated signals at δ 5.7 (s, C4, olefinic H) showing the presence of Δ^4-3-one conjugated species (261), and 5.31 (br s, C6 olefinic H) indicating the presence of unconjugated species Δ^5-3-one (264). The mass spectrum showed a complex isotopic distribution in the molecular ion region indicating loss of deuterium labelling. This mixture was, therefore, not separated for further studies.

In another set of experiments, an exactly equivalent amount of Jones reagent was added dropwise at 0°C to the solution of pregnenolone d_4 (262) in acetone, and stirring was continued for 30 minutes. The thin layer chromatography showed the presence of both Δ^4-3-one and Δ^5-3-one species, and the latter as the major product. The analysis of ions in the molecular region again indicated a complex isotopic distribution. Therefore, this procedure was also a failure towards the preparation of progesterone d_4 (261).

An attempt was made to oxidize pregnenolone d_4 (260) by the Oppenauer oxidation[90] procedure. For this all the necessary precautions were taken to ensure completely anhydrous conditions (see Experimental-II). At the end of the reaction, cyclohexanol and residual cyclohexanone were removed by steam distillation. Although the thin layer chromatography showed the complete conversion to corresponding progesterone, the mass spectrum was discouraging and showed a complex pattern of isotopic distribution. The



(263) R = H

(264) R = D

^{1}H NMR spectrum of this progesterone also showed a peak at δ 2.1, clearly indicating the loss of label from C21 carbon. Therefore, this material was also not used in further studies.

Since the removal of cyclohexanol produced during the reaction, by steam distillation required a long time, it was thought that the exchange might be occurring during steam distillation. Keeping this view, a slightly modified Oppenauer oxidation procedure[262] was used, where 2-propanol formed after the reduction of acetone (used as hydrogen acceptor) could easily be removed without taking risk and pains of steam distillation. In this reaction, aluminum t-butoxide was used instead of aluminum isoproxide. Although, 2-propanol was easily removed on a rotary evaporator, the results were again disappointing. The mass spectrum of the so obtained labelled progesterone again indicated the random loss in deuterium content.

Another experiment, with the hope that the replacement of acetone with deuterated acetone (acetone d_6) might eliminate the exchange, was undertaken. The pregnenolone d_4 (260) was, once again, oxidized by the procedure described by Oppenauer using aluminum t-butoxide as a catalyst and acetone d_6 as hydrogen acceptor. Progesterone was formed but unfortunately it was of no use for further studies. The examination of the molecular ion region in the mass spectrum indicated the increased abundances of d_5 and d_6 species showing an increased deuterium content due to incorporation of some more deuterium atoms. This attempt was another failure towards the synthesis of progesterone-d_4 (261).

All these attempts forced us to believe that the chemical methods available for the direct oxidation of pregnenolone d_4 (260) to progesterone

d_4 (261) without losing or increasing its deuterium content were unsatisfactory. However, some micro-organisms can oxidize pregenolone (214) to progesterone (213) in one step. Pregnenolone can be oxidized to progesterone in 82% yield Micrococcus dehydrogenas, or by Bacillus pulvifaciens in 30% yield (Fig. 123).

Attempts towards the synthesis of progesterone d_4 (261) by chemical methods continued and the Scheme III was proposed (Fig. 124).

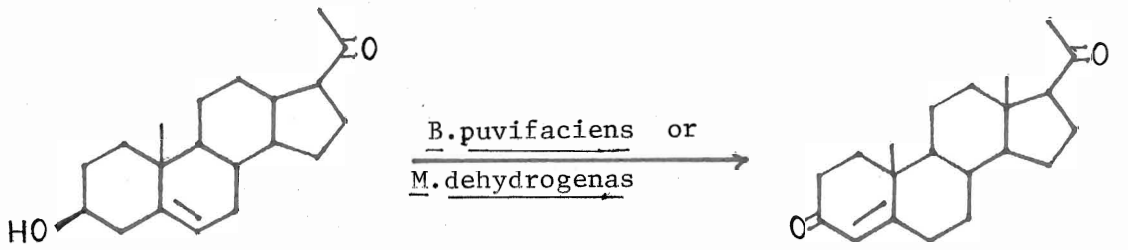
The initial step in this envisaged Scheme III was the preparation of Δ^5pregnene-3,20-dione-3,20-bis-ethylene dioxyketal (progesterone-bis-ketal) (265). Allen et al.[263] in 1954 have synthesized it in 80% yield and their procedure was adopted in the present study.

The slow distillation of the reaction mixture containing progesterone (213) in ethylene glycol and catalytic amount of p-toluenesulfonic acid, over a period of 2-3 hours at 1.5 mm Hg pressure afforded a pure sample of progesterone-bis-ketal (265).[263] The infrared spectrum of this compound showed no absorption band in the carbonyl region, and other spectral data were in agreement with the structure.

The next step in the Scheme III, was the selective hydrolysis of C20 ketal group from the bis-ketal (265).

Hirsch and Fujimoto[264] have reported a very successful method for the selective hydrolysis of a 20-ketal during the synthesis of 21-hydroxymethylprogesterone. This selective hydrolysis was achieved in 98% yield by stirring a solution of progesterone bis-ketal (265) in water saturated benzene containing a required amount of ethereal solution of p-toluenesulfonic acid, for 1.5 hours at room temperature.

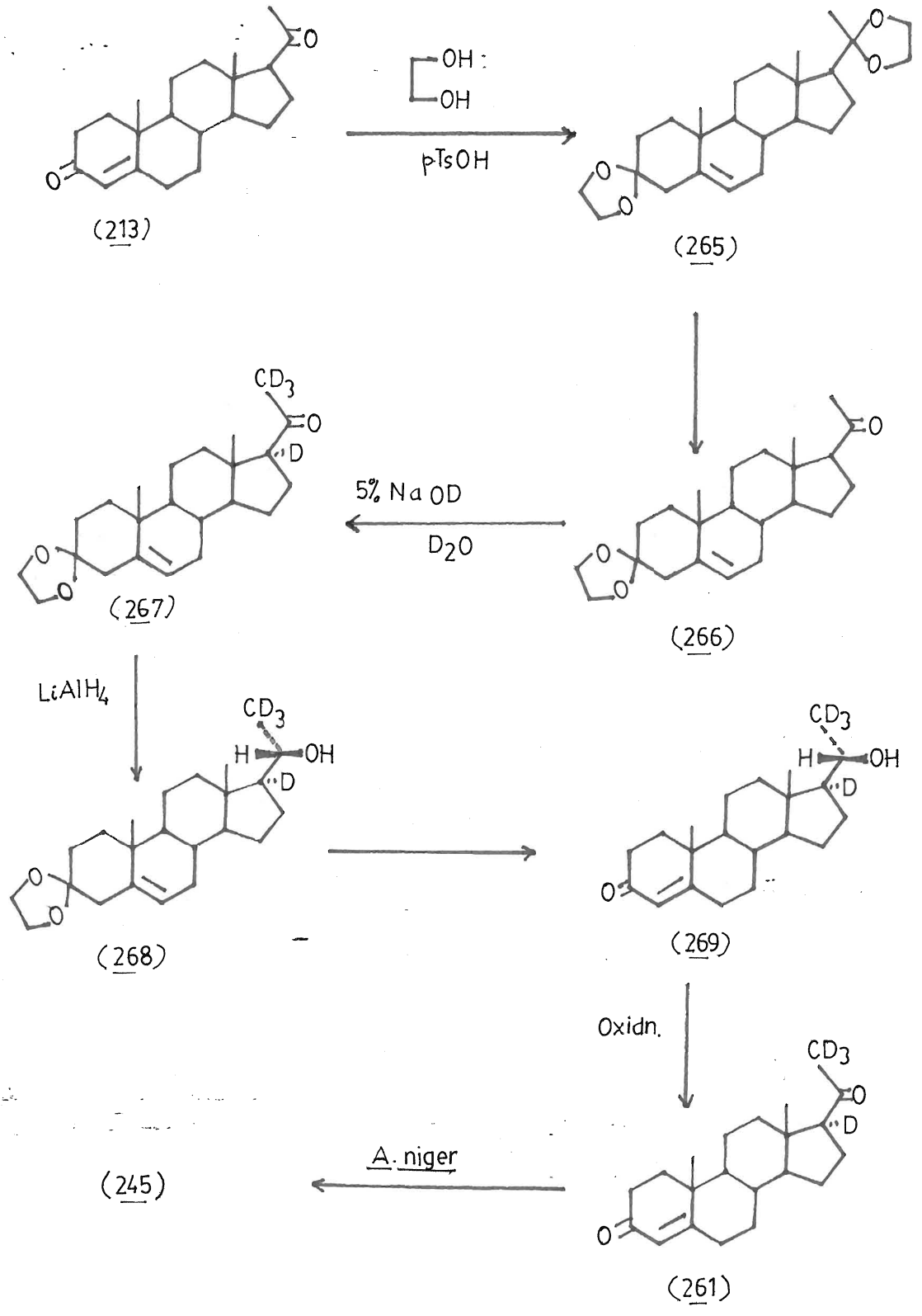
Figure 123



(214) (213)

Figure 124

Scheme-III



The infrared spectrum of the above product, Δ^5-pregnene-3,20-dione-3-monoethylenedioxy ketal (266) showed an absorption band at 1700 cm^{-1} for the C20 carbonyl functionality, and other spectral data were also consistent with the structure (266).

Sondheimer et al.[280] reported the synthesis of progesterone-3-monoethylene ketal (266) in one step from progesterone (213), but the yield was only 25%, and therefore, it was worthwhile to make the bis-ketal, then hydrolyze it selectively to the monoketal (266).

The next step in the Scheme III was the incorporation of deuterium atoms in the monoketal (266) at C17 and C21 positions. For this purpose, a procedure similar to that used in the labelling of pregnenolone was employed, and the expected product Δ^5-pregnen-3,20-dione-3-ethylenedioxy ketal-17,21,21,21-d_4 (267) was obtained in good yield. The ^1H NMR spectrum of this compound showed no signal between δ 2.0 and 2.5 indicating the absence of any hydrogen atoms at either C17 or C21 positions. The ^2H NMR spectrum indicated signals at δ 1.88 (for C21 CD_3) and 2.15 (for C17 ^2H), which were not well resolved. The mass spectrum indicated the molecular ion at m/z 362 (4.3%) and the analysis of the ions at m/z 364-360 gave the following molecular isotopic composition: d_4 97.68%, d_3 3.02%, $d_2 = d_1 = 0\%(\pm 1)$.

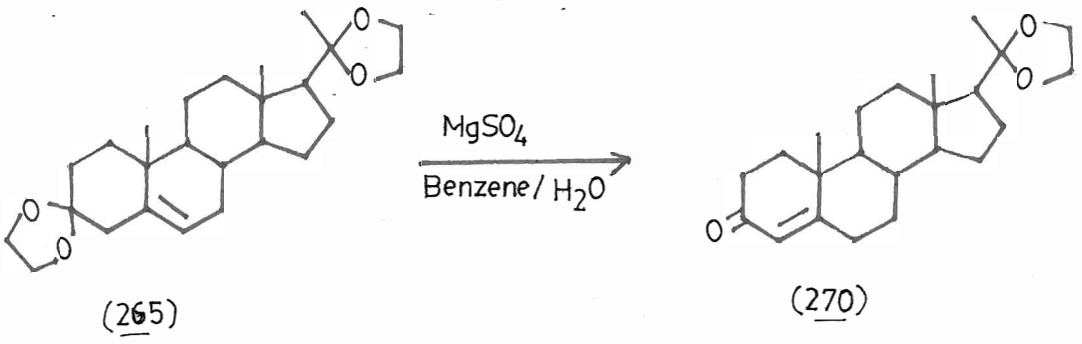
The progesterone-3-monoethylene ketal-d_4 (267) was satisfactorily labelled and was therefore used in the next step. After the successful labelling at C17 and C21 positions in the progesterone-3-monoethylene ketal, hydrolysis of the remaining ketal group at C3 position was desired to get progesterone d_4 (261). A very mild hydrolysis condition was desired to avoid any deuterium exchange in the product.

Brown et al.[281] have reported the selective hydrolysis of progesterone-bis-ketal (265) to progesterone-20-monoethylenedioxy ketal (270) using magnesium sulfate in water saturated benzene. Even after three days stirring the solution of progesterone-3-monoketal-d_4 (267) in water saturated benzene containing a suspension of magnesium sulfate, did not show any sign of hydrolysis (Fig. 125). Since the time required for the complete hydrolysis using this procedure may be very long and may lead to deuterium loss in that interval, no more attempts were made using this procedure.

A tlc spot from the three days old solution of progesterone-3-monoketal-d_4 (267) in $CDCl_3$ (used for ^1H NMR) indicated an UV active compound which corresponded with authentic progesterone. The hope that chloroform may be sufficiently acidic to hydrolyze ketal group, a solution of progesterone-3-monoketal-d_4 (267) in chloroform saturated with water was stirred at toom temperature, but no significant amount of hydrolyzed product was formed.

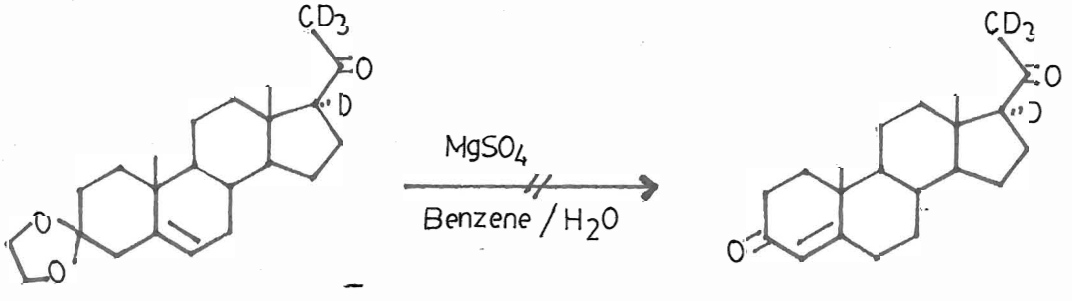
Any further attempt to hydrolyze 3-ketal group of (267) in the presence of 20-keto group was abandoned. The milder conditions for such a hydrolysis will require longer time, long enough maybe to cause exchange of deuterium at the enolizable positions. On the other hand, stronger acidic conditions such as using BF_3 or HCl, will exchange deuterium faster due to the rapid enolization at the labelled positions.

Therefore, it was desirable to reduce 20-keto group of progesterone-3-monoethylenedioxy ketal-d_4 (267) to 20-OH, so that the hydrolysis of C3 ketal will no longer cause any exchange of deuterium.



(265) (270)

Figure 125



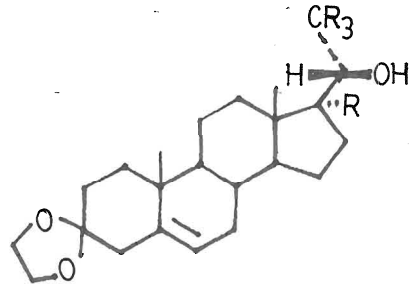
(267) (261)

Cainelli et al.[266] have reported a very efficient conversion of Δ^5-pregnene-3,20-dione-3-monoethylenedioxy ketal (266) to Δ^5-pregnene-3-one-20β-ol-3-ethylene dioxyketal (271). Therefore, the treatment of Δ^5-pregnene-3,20-dione-3-ethylene dioxyketal-17,21,21,21-d_4 (267) in dry THF with lithium aluminum hydride afforded Δ^5-pregnene-3-one-20β-ol-3-ethylene ketal-17,21,21,21-d_4 (268) slightly contaminated by Δ^5-pregnene-3-one-20α-ol-3-ethylene ketal-17,21,21,21-d_4 (273) (about 5%). The separation of these two isomers was not required and the mixture was directly used in the next step without further purification.

The 3-keto group was, then, regenerated by hydrolysing the 3-ketal group using a few drops of 36% HCl solution in chloroform.[269] The crude product, thus obtained, showed the presence of two isomers, Δ^4-pregnene-3-one-20β-ol-17,21,21,21-d_4 (269) (about 95%) and Δ^4-pregnene-3-one-20α-ol-17,21,21,21-d_4 (276) (about 5%). After two crystallizations, a pure sample of the 20β isomer (269) was obtained and identified with the authentic unlabelled material (274).

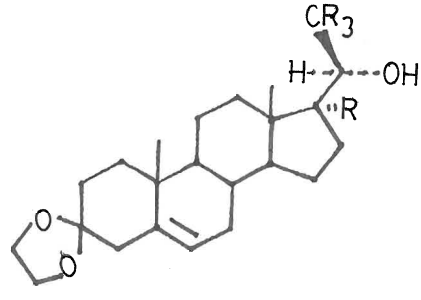
The infrared spectrum of (269) had an absorption band at 1680 cm^{-1}, for a conjugated carbonyl group. The mass spectral analysis of ions at m/z 322-318 showed the molecular isotopic composition of d_4 94.80%, d_3 5.19% $d_2 = d_1 = d_1$ 0% (±1%).

The last step towards the synthesis of progesterone-d_4 (261), in the envisaged Scheme III, was the oxidation of C-20 hydroxyl group to carbonyl, and was readily achieved by Jones oxidation of (269). The molecular isotopic composition of so obtained progesterone-d_4 (261) was d_4 85.8%, d_3 6.4%, d_2 0%, d_1 4.8%, d_0 3.9%, indicating that there had been some



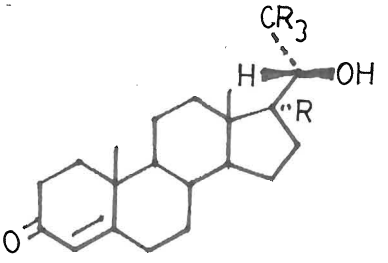
(271) R = H

(268) R = D



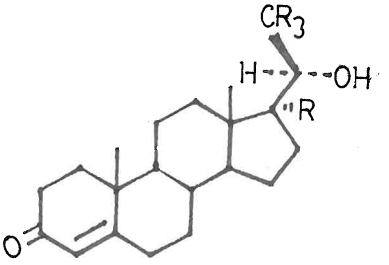
(272) R = H

(273) R = D



(274) R = H

(269) R = D



(275) R = H

(276) R = D

exchange during the oxidation. The ^2H NMR spectrum of the above product indicated signals at δ 2.17 (s, C9 1^2H, C17 ^2H) and 1.90 (C9 3^2H, C21-CD_3 group).

Incubations with Aspergillus niger ATCC 9142

In spite of several available chemical methods[282,283] for the synthesis of deoxycorticosterone (205) from progesterone (Fig. 126) and pregnenolone (Fig. 127), they were not well suited for the synthesis of labelled deoxyprogesterone having an appreciable amount of labelling at C17 and C21 positions. The disadvantage associated with these methods were the involvement of at least one step in the synthetic sequence, drastic enough to cause loss in the deuterium content of the desired product.

Microbial steroid hydroxylations[284] have been known for many years. The inherent advantages with enzymatic hydroxylations are their specificity and stereoselectivity. The fungus Aspergillus niger has been reported[285] to hydroxylate progesterone, 6β-, 11α-, 11β- and 14α-hydroxyprogesterone, at the C21 position specifically. And therefore, Aspergillus niger ATCC-9142 was used as a 21-hydroxylator on progesterone d_4 (261) to prepare deoxycorticosterone d_3 (245).

It has been proposed[286] that at the C2, C6, C17 and C21 positions of Δ^4-3,20-diketosteroids, the carbon to be hydroxylated is first activated by enolization and then hydroxylation occurs by electrophilic attack of oxygen at the enol (Fig. 128). However, studies by Holland et al.[269] using the fungus Aspergillus niger ATCC 9142, a C21 hydroxylator progesterone 21-d (277) as a substrate did not support the above proposal of enzyme

Figure 126

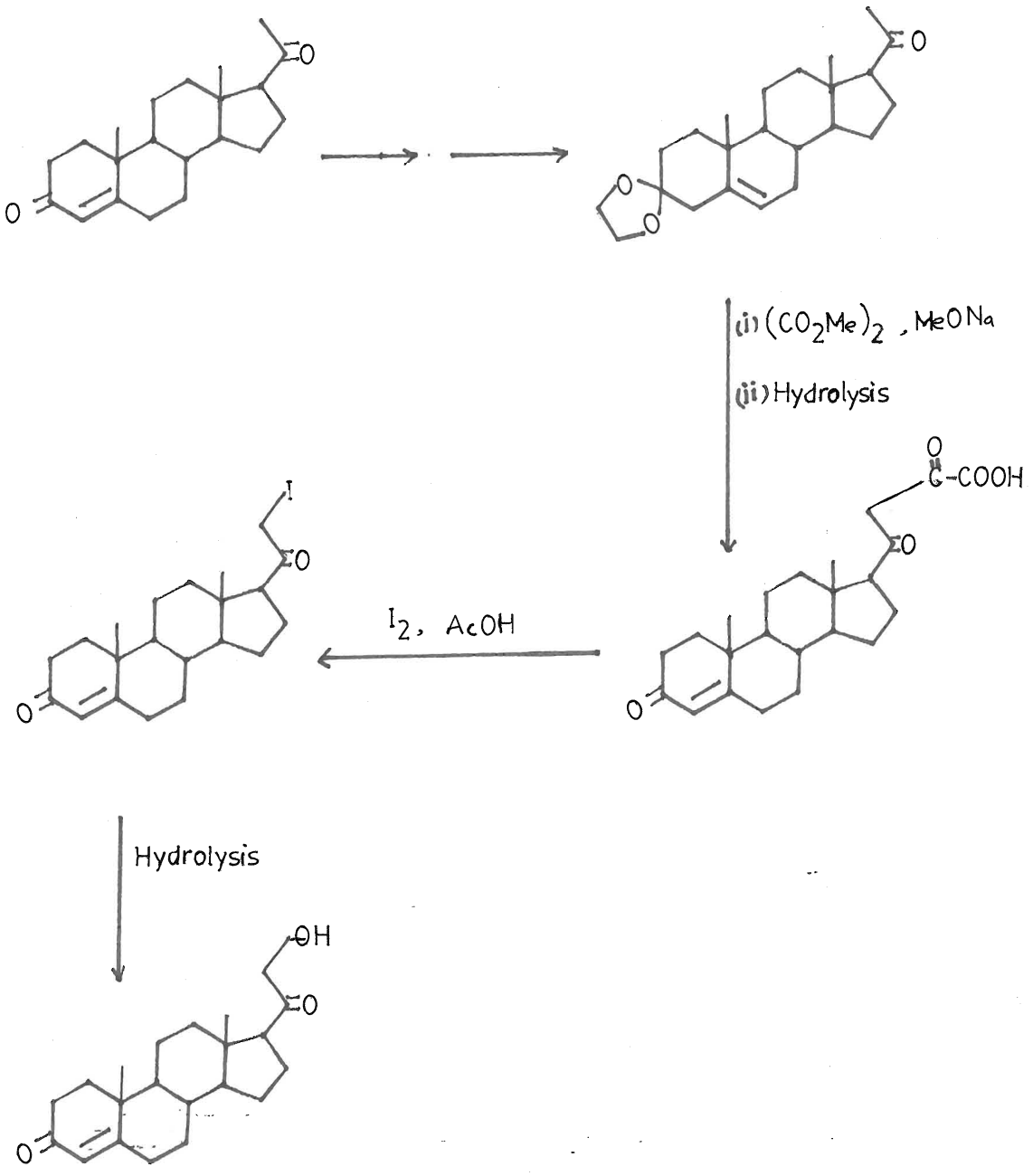
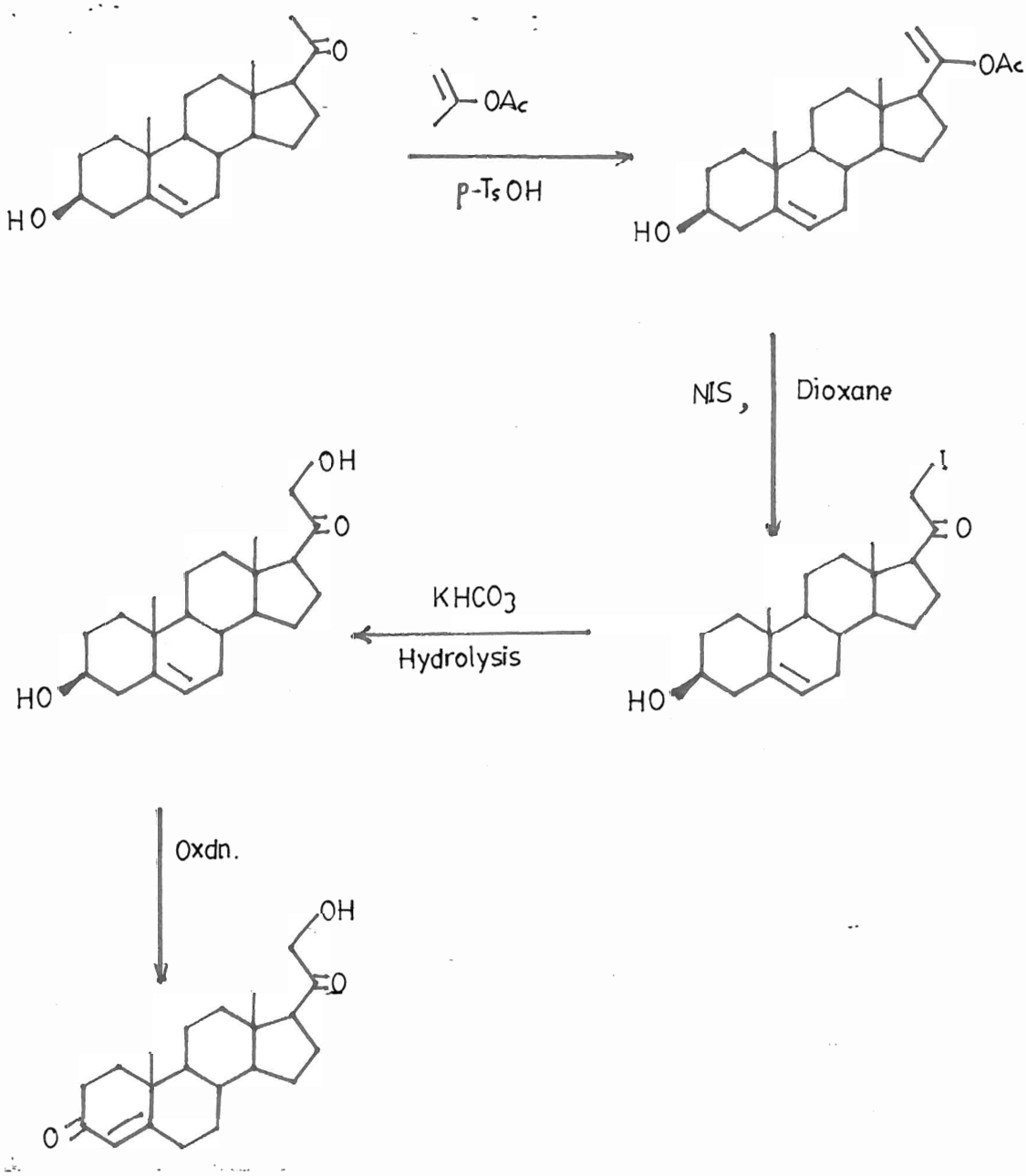
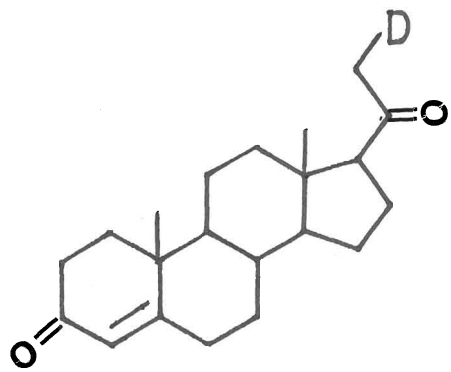


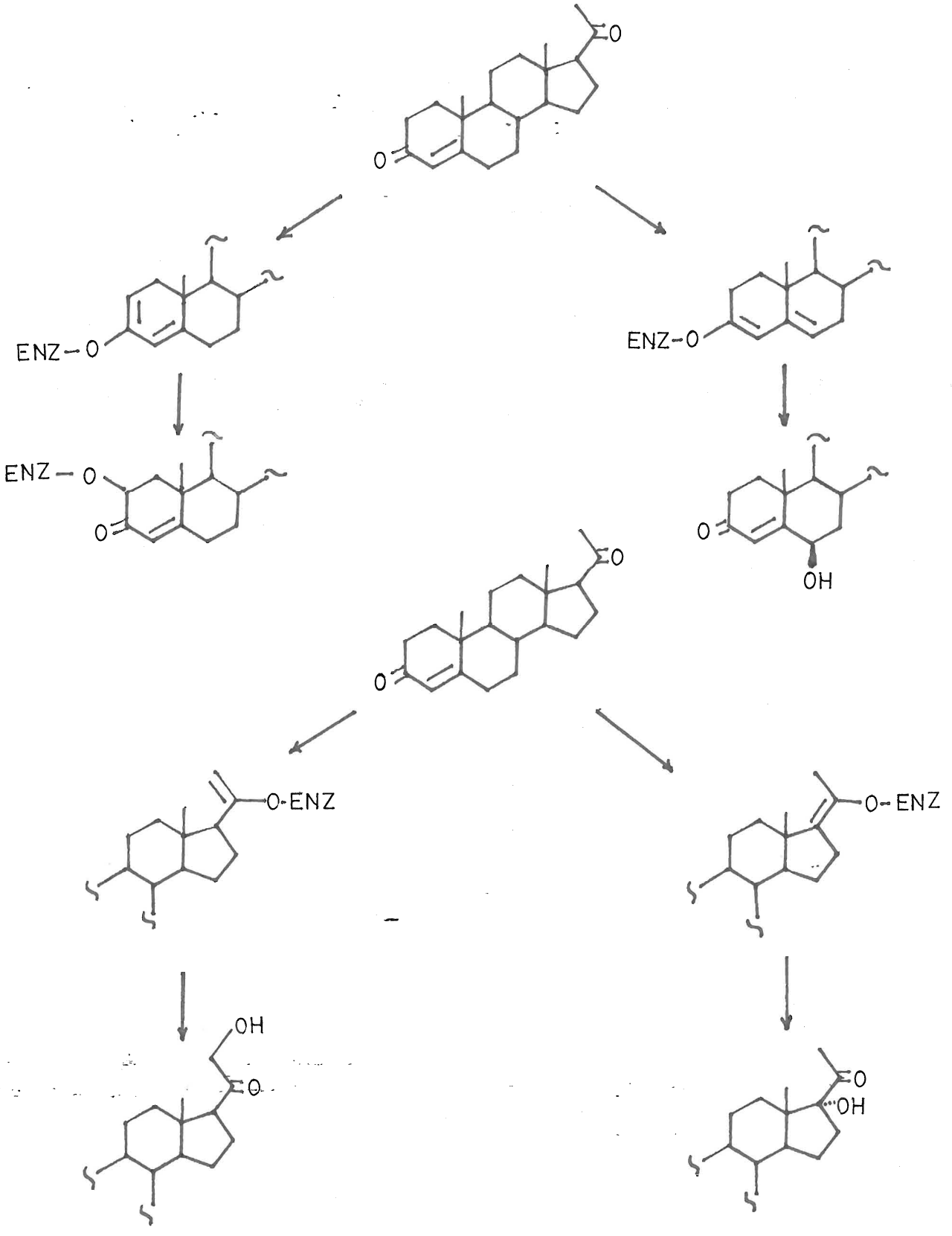
Figure 127


HO
O
OAc
p-Ts OH
OAc
HO
NIS, Dioxane
I
O
HO
KHCO3
Hydrolysis
OH
O
HO
Oxdn.
OH
O
O



(277)

Figure 128



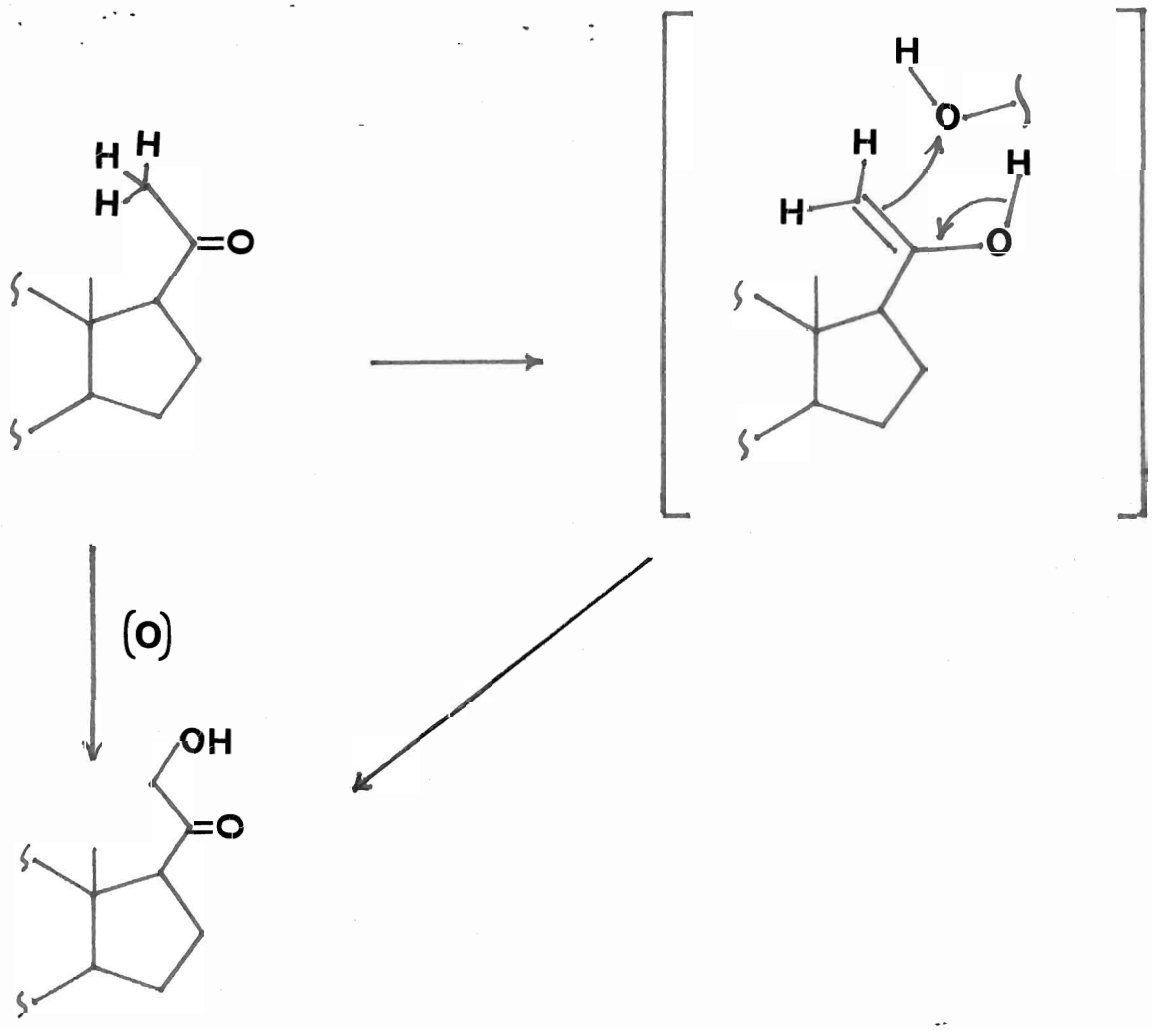
participation in enol formation during the 21-hydroxylation. They suggested that the enzymatic C21 hydroxylation involve the direct insertion of oxygen into a C21 carbon-hydrogen bond in the rate determining step and certainly not one involving the reversible C20-C21 enolization of the C20 carbonyl group (Fig. 129). This suggested that the desired labelled deoxycorticosterone-d_3 (245) may be obtained without losing any significant amount of deuterium from the labelled progesterone-d_4 (261) using A. niger as a C21 hydroxylator.

Previous findings,[269] showed that there was no exchange of deuterium with protium from medium by enolization as the recovered labelled progesterone had an isotopic distribution identical with starting material.

Incubation of (261) with the fungus Aspergillus niger yielded about 10-15% labelled deoxycorticosterone. The labelled deoxycorticosterone obtained from these incubations had suffered some deuterium loss. Several incubations were performed and the best sample of deoxycorticosterone had only half of the original deuterium, distributed equally at the C21 positions. This was evident from its ^2H NMR spectrum at both 13.8 MHz and 61.4 MHz, indicating signals at δ 1.83 (s, ^2H, C17 ^2H) and two signals of equal integration at δ 3.87 (s) and 4.0 (s) for two diastereotopic deuteriums at C21 position.

The mass spectrum of the labelled deoxycorticosterone obtained from the above incubation showed a complex pattern in the molecular ion region. The inherent problem with the mass spectrum of even unlabelled deoxycorticosterone (205) was the very weak molecular ion and significant abundance of a M + 1 ion. The mass spectra were obtained at different

Figure 129



Alternate pathways for the C-21-hydroxylation of C-20 ketosteroides.

ionization voltages, but the problem remained the same. HPLC was also employed to purify the labelled deoxycorticosterone on reverse phase column. The preparation scale purification on small reverse phase column was a tedious job, however, the silica gel columns used for the purpose gave only poor resolutions.

A comparatively better yield (20%) of labelled deoxycorticosterone (245) was obtained on incubation of the substrate after replacing the medium with distilled water. The loss of deuterium was also observed in these incubations. On the other hand, the recovered progesterone d_4 (261) did not show any significant exchange of deuterium with protium from medium. This observation was in agreement with Holland and Auret's findings.[269]

This suggested that exchange with the medium occurred only after the product deoxycorticosterone-d_3 (245) was released into the medium.

This exchange phenomenon prompted the measurement of pH at the end of incubations with unlabelled progesterone (213) and efforts to neutralize the acidity of the medium. The results of these pH measurements after four days of substrate incubations are as follows:

1. Incubation in growth medium (Czaped Dox nutrient) pH 2.3
2. Incubation after replacing the growth medium with distilled water (150 mL) pH 2.5
3. Incubation after replacing the growth medium with buffer pH 6.86 (3.5 g/100 mL) pH 3.3

No conversion of progesterone was observed when incubation was done after replacing the medium with distilled water (200 mL) and suspending 5 g of calcium carbonate in it. In all the replacement incubations,

mycelia were washed with distilled water prior to its resuspension in the desired system. The above pH values showed that the media were sufficiently acidic to cause the loss of deuterium from the labelled deoxycorticosterone (245).

This exchange of label with medium is in contradiction with the previous report,[269] where no such loss of label was observed. The difference in the incubation conditions may be accounted for by these different observations. All the present incubations were performed in one Liter Erlenmeyer flasks on New Brunswick rotary shaker at about 180 rpm at room temperature, while the previous workers[269] performed it in a 5-L fermentation vessel, mechanically stirred and continuously aerated with sterilized air at 28°C.

It is now well established that the oxygen atom in the C21 hydroxyl group originates from molecular oxygen[286] (Fig. 130) after being activated through a complicated sequence (Fig. 131).[287] Therefore, continuous passage of sterilized air through the incubation vessel may be providing conditions of comparatively higher pH (≥5.0),[288] or more efficient transformation.

Once the labelled deoxycorticosterone (245) is released into the significantly acidic medium, it may rapidly undergo reversible enolization towards C20-C21 and subsequently suffers the loss of deuterium (Fig. 132). The loss of deuterium in the product was only from C21 position as indicated by ^{2}H NMR spectrum and mass spectrum (showing an abundant fragment ion at m/z 272 formed by the loss of side chain and retention of deuterium at the C17 position). This is supported by the fact that the enolization towards C20-C21 position is favoured kinetically (Fig. 133).[289]

Figure 130

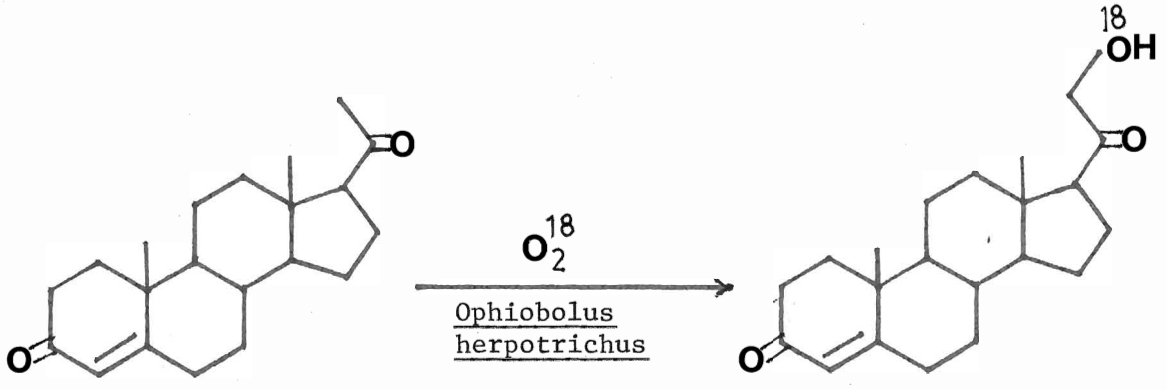
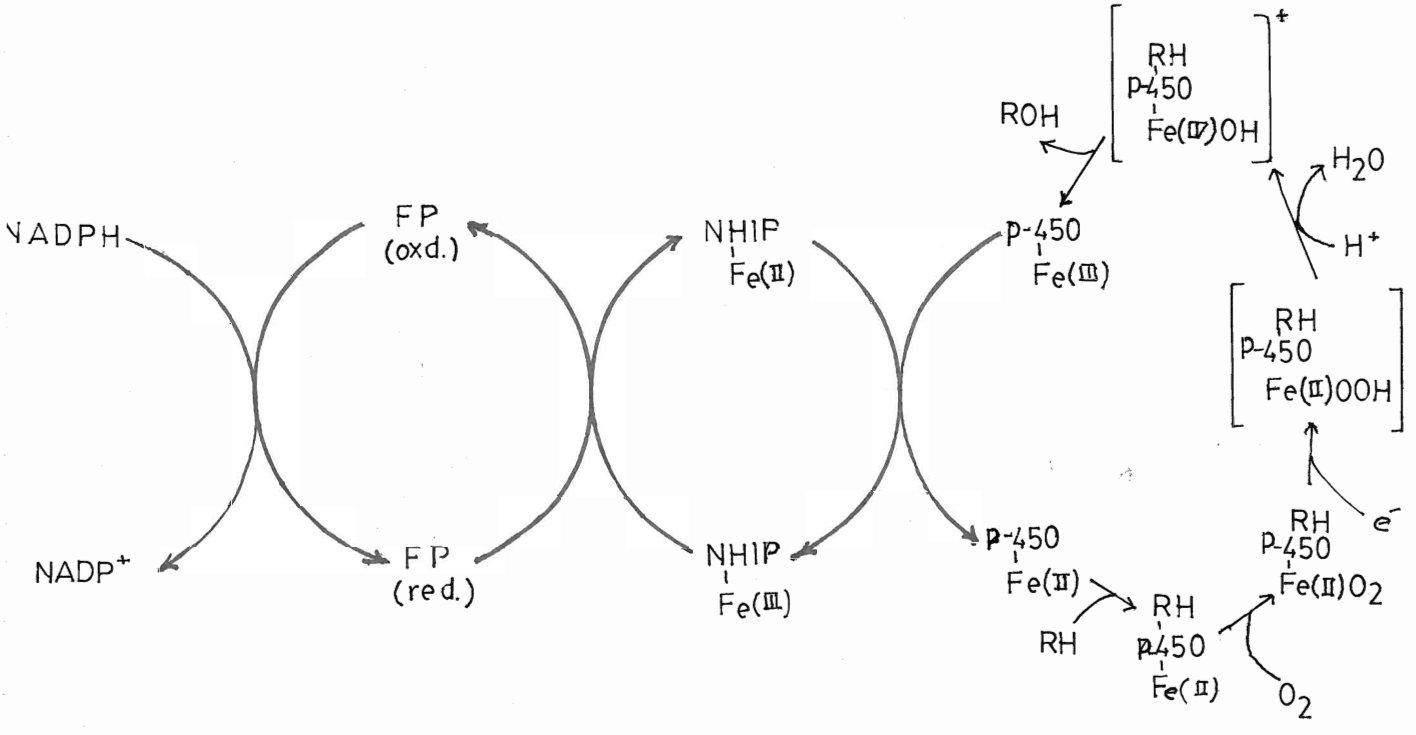


Figure 131



Proposed metabolism of steroid hydroxylation.

FP,adrenodoxin reductase; NHIP,adrenodoxin; RH,steroid, p-450,cytochrome p-450

Figure 132

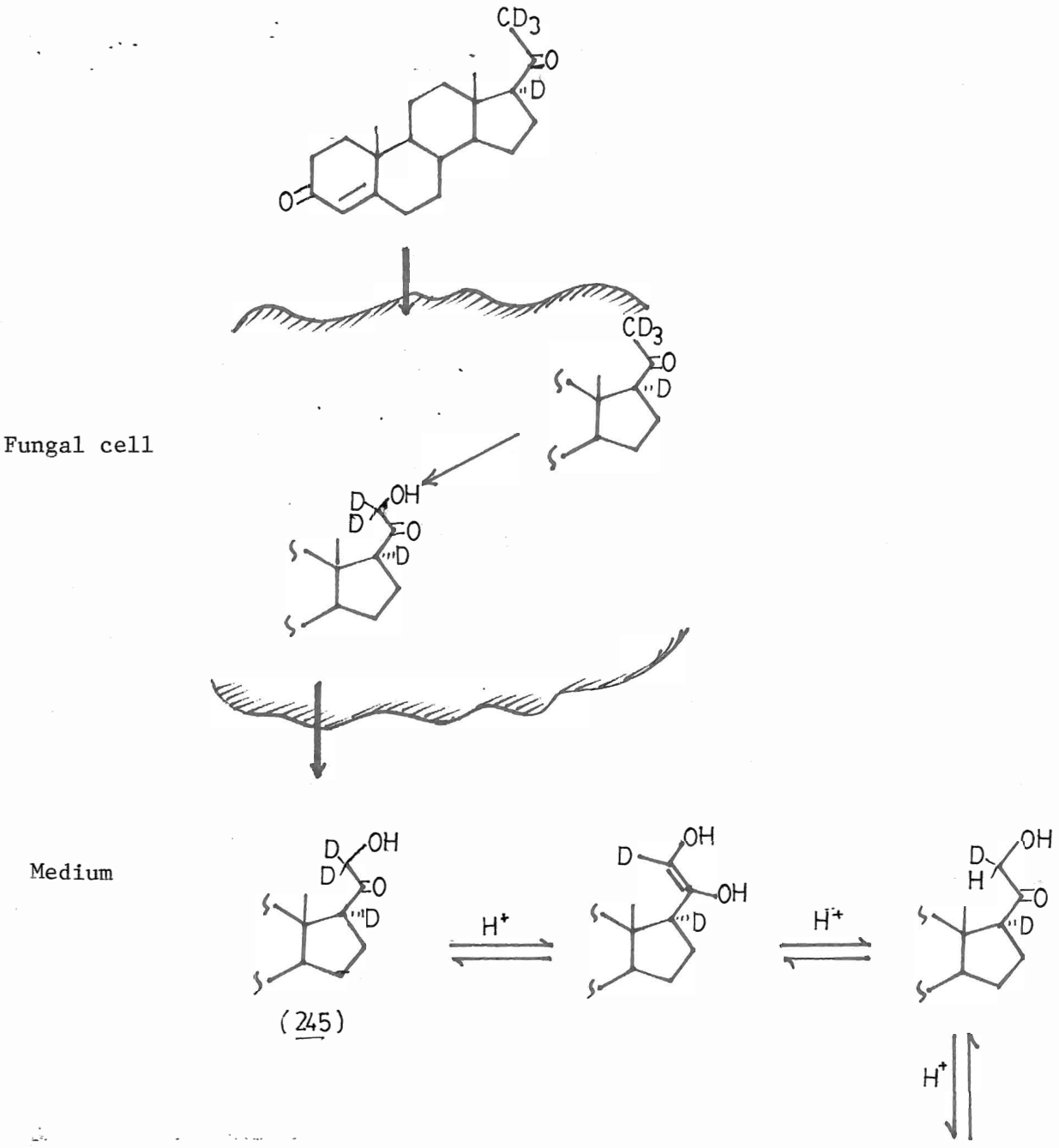
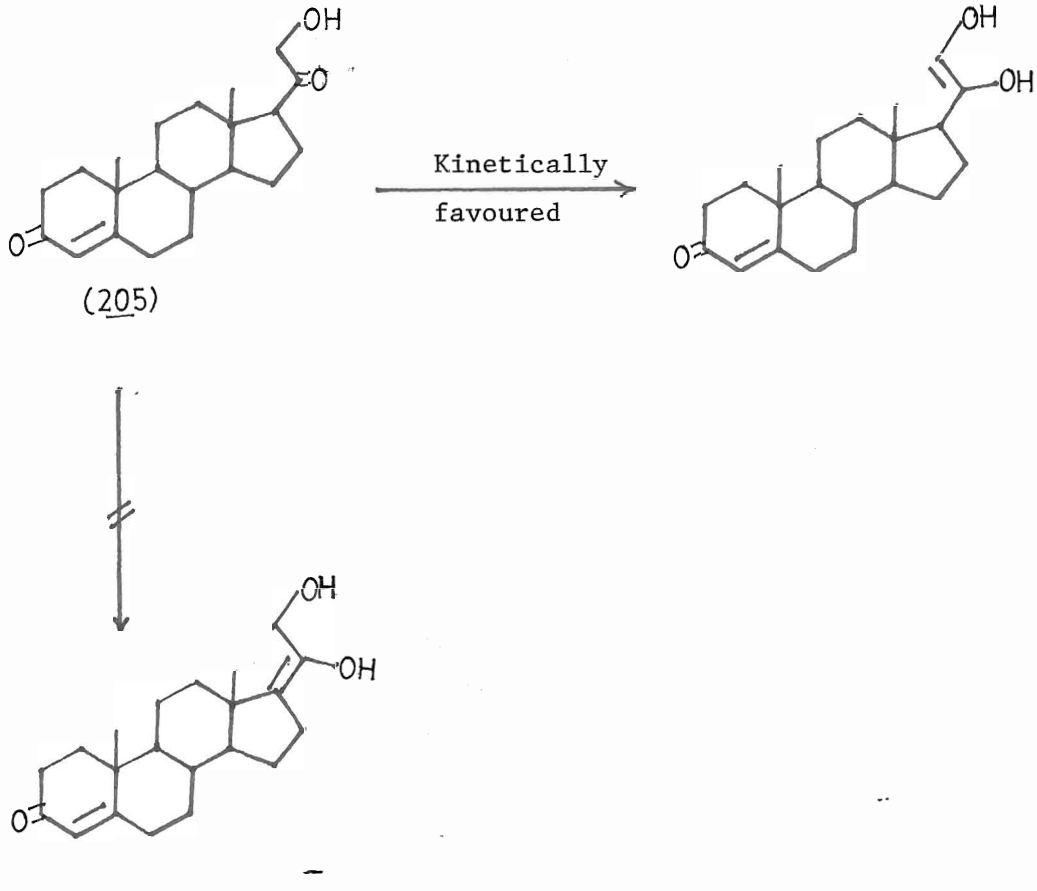


Figure 133



The lowering of the pH of medium was probably caused by the release of carboxylic acids produced by the metabolism of carbohydrates present in the growth medium. Since the simpler carbohydrates are more easily metabolized, incubation studies were directed using higher carbohydrates and a more complex growth medium.[290] It was hoped that this would slow down the release of carboxylic acids into the medium, and that the pH of the medium may therefore not go so low.

The use of sucrose, peptone and corn steep liquor combination as a growth medium (growth medium #2) for Aspergillus niger gave good results only from the transformation point of view (about 60% conversion of progesterone). In these incubations, the pH also dropped low enough to cause exchange. Therefore, the incubation of progesterone-d_4 (261) was not performed using this growth medium.

The pH measurements recorded in this set of incubations are as follows:

1. After three days of mycelium growth — pH 4.3
2. pH of the medium at the time of extraction
   (i.e., after 4 days of incubation)
   (a) Incubation in the growth medium #2 — pH 2.7-2.9
   (b) Incubation after replacement of medium with distilled water — pH 2.5

A comparatively more complex growth medium (growth medium #3) was used in the final set of incubations with Aspergillus niger. This medium consisted of malt extract, peptone and corn steep liquor. After three days of mycelium growth, the medium had a pH of 4.2, which dropped to 3.2 on the fourth day.

The pH measurements at the time of product extraction are as follows:

1. Incubation in the growth medium. pH 2.8
2. Incubation after the replacement of the medium with distilled water pH 2.8

These observations show that even in this medium, the pH is sufficiently low to cause the loss of label. Another handicap with this medium was the low yield of deoxycorticosterone, which was not more than 20% in both the above incubations.

The micro-organism which could perform the 21-hydroxylation of progesterone specifically, near neutral pH is now desirable.

McAleer and Dulaney[291] reported the 21-hydroxylation of progesterone in 35-45% yield by the micro-organism Wojnowicia graminis. Meystre et al.[292] demonstrated the 21-hydroxylation of progesterone with Ophiobolus herpotrichus is 50-80% yield. This fungus has an advantage of growing at pH 6.8 which is suitable for the synthesis of deoxycorticosterone-d_3 (245). Both the micro-organisms have been recently imported and the incubations are in progress.

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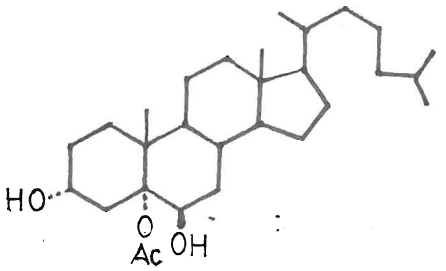
APPENDIX

During the preparation of this manuscript, structures of 3β,5β-oxidocholestan-6α-ol (134) and 3β,5β-oxidocholestan-6α-ol-6-acetate (131) were re-investigated. The 100.16 MHz ^{13}C NMR spectrum of (134) recently recorded on the Bruker WH-400, was found to be more consistent with the structure 3α,5α,6β-cholestanetriol (133) (Table 16), although the melting point was not in agreement with the literature.[97] This led us to re-investigate the structure of the oxetane acetate (131), which also was found to be consistent with 3α,5α,6β-cholestanetriol-5-acetate (278) (Table 16). This structure (278) can also explain the ^{1}H NMR spectrum, previously assigned to the oxetane acetate (131), as follows: δ 4.65 (br s, 1H, C6 Hα), 4.2 (br s, 1H, C3 Hβ), 2.85, 2.15 (d, d, AB pattern, 2H, C4 methylene group), 0.66 (s, 3H, C18 methyl group), 1.13 (s, 3H, C19 methyl group) and 2.0 (s, 3H, C5 acetate). This also explains the absorption band at 3500 cm^{-1} in the infrared spectrum of (131) and the elemental analysis (calculated for $C_{29}H_{50}O_3$, C 75.35, H 10.72; found, C 75.35, H 10.82). However, the x-ray structure is desirable for correct assignment.

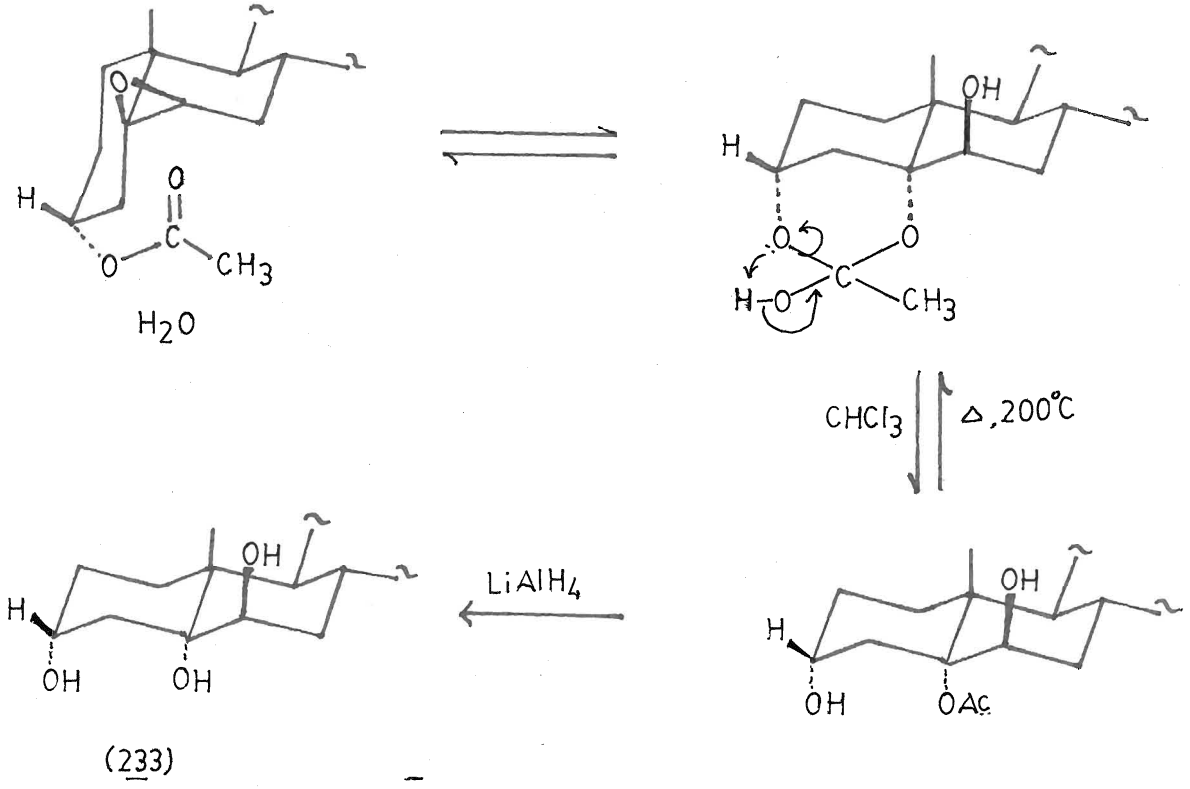
The probable mechanism for the formation of 3α,5α,6β-cholestanetriol-5-acetate (278) from 3α-acetoxycholestan-5β,6β-epoxide (130) and their interconversions are represented in Figure 134.

The allylic alcohols, Δ^5-cholesten-4α-ol (204) and Δ^5-cholesten-4β-ol (200) have been observed to undergo dehydration, yielding $\Delta^{4,6}$-cholestadiene



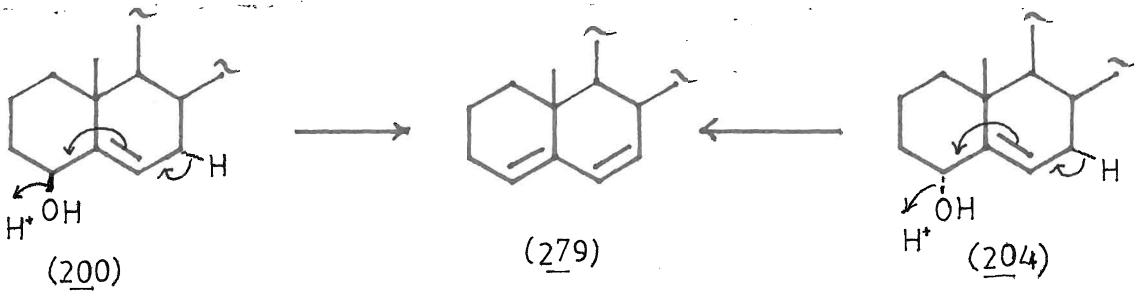
(278)

Figure 134



(233)

Figure 135



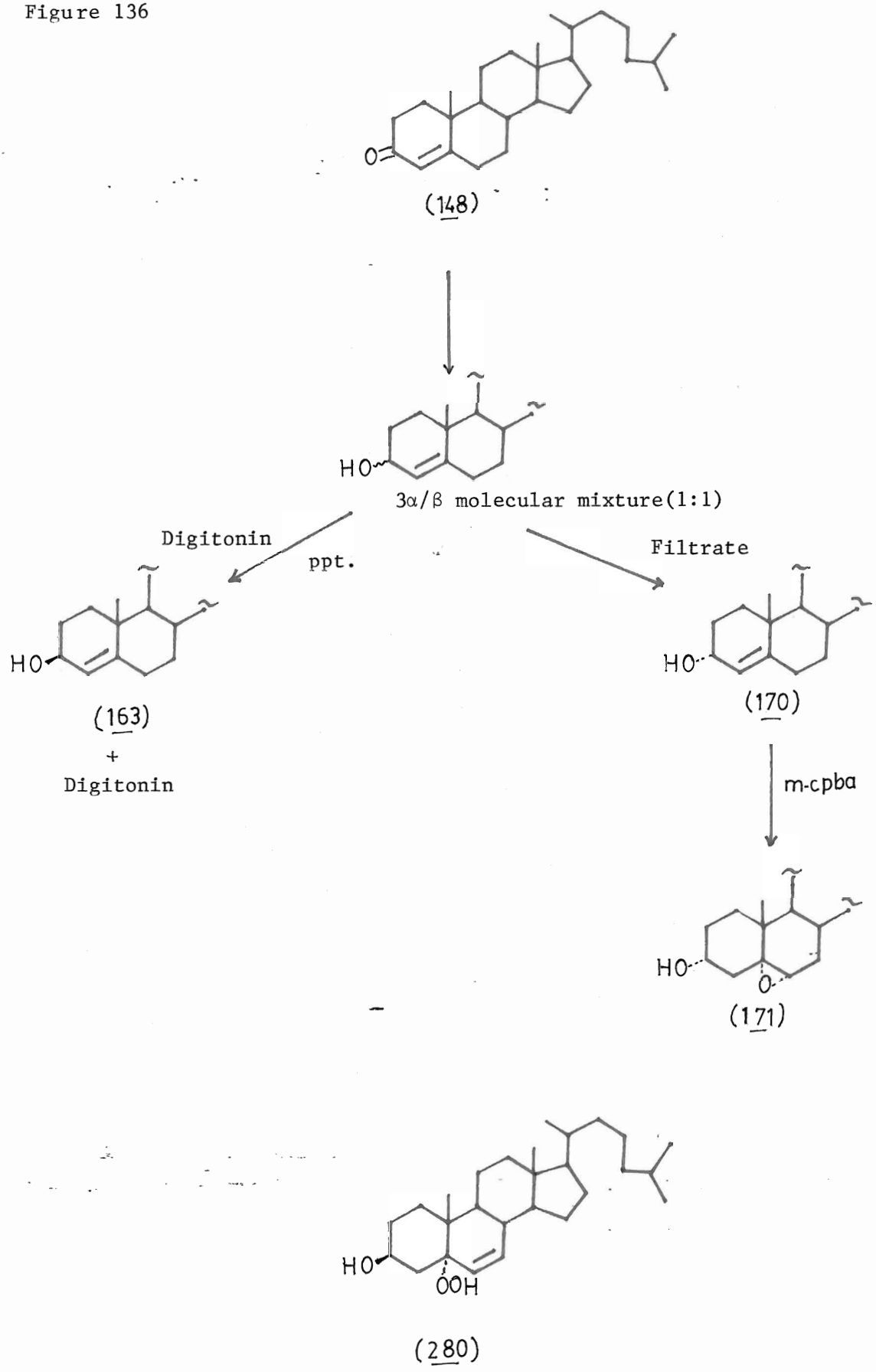
(200) (279) (204)

(279). This was indicated when their 100.16 MHz ^{13}C NMR spectra (Table 13) were recorded from samples which for two months were in solution ($CDCl_3$). Chloroform being slightly acidic may affect this dehydration. The probable mechanism for this dehydration is presented in Figure 135.

The remaining member of the 4,5-epoxides series, 4α,5α-epoxycholestan-3α-ol (171) has been prepared also, during the preparation of this manuscript. For this purpose, the desired Δ^4-cholesten-3α-ol (170) was was prepared using the procedure described by Schoendeimer and Evans.[299] The cholestenone (148), on reduction with aluminum isopropoxide, yielded a 1:1 mixture of Δ^4-cholesten-3α-ol (170) and Δ^4-cholesten-3β-ol (163). The resolution of these two isomers was effected by selective precipitation of the β-isomer (163) with digitonin. Δ^4-Cholesten-3α-ol (170) on epoxidation with m-chloroperbenzoic acid yielded the desired epoxide (171) (Fig. 136). The epoxidation, as expected, occurs only from the α-face of the olefin because of the directing effect of the 3α-hydroxy group.

The ^{13}C NMR spectrum[300] of Δ^6-cholesten-5α-hydroperoxy-3β-ol (280) (Table 14), a well characterized compound, was used in the assignment of the ^{13}C NMR spectrum of Δ^6-cholesten-3β,5α-diol (186).

Figure 136


O
(148)
HO
3α/β molecular mixture(1:1)
Digitonin
ppt.
Filtrate
HO
(163)
+
Digitonin
HO
(170)
m-cpba
HO
O
(171)
HO
OOH
(280)